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Introduction to General Field Procedures (Field Manual of Wildlife Diseases)

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Section 1

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Dissecting a bird at the National Wildlife Health Center
Photo by Phillip J. Redman

Introduction to General Field Procedures

"Given the conspicuous role that diseases have played, and in many parts of the world continue to play, in human demography, it is surprising that ecologists have given so little attention to the way diseases may affect the distribution and abundance of other animals and plants. Until recently, for example, ecology textbooks had chapters discussing how vertebrate and invertebrate predators may influence prey abundance, but in most cases you will search the index in vain for mention of infectious diseases." (May)

A basic premise for the preparation of this Manual is that disease in free-ranging wildlife is of concern and that disease prevention and control are desirable actions. However, these are not universally held perspectives. There are those who when confronted with disease outbreaks in free-ranging wildlife ask — "Why bother?" Also, the same individuals who may reject the need for response to one situation may demand a response to another situation. We acknowledge in this Manual the existence of this question by making reference to it, but we do not offer a direct response. To do so would require this Manual to address the full spectrum of individually held values, perspectives, interests, and beliefs within human society that form the basis for the underlying issues which create the question of "why bother?" Those factors would also need to be addressed within a context of the different roles and responsibilities of public agencies, and would need to include some additional considerations. Such an undertaking is outside the scope and purpose of this Manual. Although no direct response is offered, readers will gain considerable information regarding disease occurrence and impacts in the chapters that follow. This information should be of value in assisting readers to address the questions of "why bother?" from their own set of values and interests.

Section 1 of the Manual provides basic information regarding general field procedures for responding to wildlife disease events. Field biologists provide a critical linkage in disease diagnostic work and greatly affect the outcome of the laboratory efforts by the quality of the materials and information that they provide. The chapters in this section are oriented towards providing guidance that will assist field biologists in gathering the quality of information and specimens that are needed. Readers will find information regarding what to record and how; guidance for specimen collection, preservation, and shipment; and how to apply euthanasia when such actions are warranted. Disease operations are managed at the field level and they can be aided by general preplanning that can be utilized when disease emergencies arise; therefore, contingency planning is included within the Disease Control Operations chapter. Disease control techniques, including equipment that is used, are the main focus for this highly illustrated chapter. Section 1 is concluded with a chapter about the proper care and use of wildlife in field research. The guidelines provided address the continual need to consider animal welfare in all aspects of wildlife management.

Quote from:

May, R.M., 1988, Conservation and disease: Conservation Biology, v. 2, no. 1, p. 28–30.

Chapter 1

Recording and Submitting Specimen History Data

History can be defined as a chronological record of significant events. In wildlife disease investigations, determining the history or background of a problem is the first significant step toward establishing a diagnosis. The diagnostic process is often greatly expedited by a thorough history accompanying specimens submitted for laboratory evaluation. This information is also important for understanding the natural history or epizootiology of disease outbreaks, and it is difficult, if not impossible, to obtain the history after the outbreak has occurred. Detailed field observations during the course of a die-off and an investigation of significant events preceding it also provide valuable information on which to base corrective actions. The most helpful information is that which is obtained at the time of the die-off event by a perceptive observer.

What Information Should Be Collected

What seems irrelevant in the field may be the key to a diagnosis; therefore, be as thorough as possible. Avoid preconceptions that limit the information collected and that may imperceptibly bias the investigation. A sample specimen history form, which lists some categories of information that are helpful, is in Appendix A. A good description of unusual behavior or appearance, if any, an accurate list of what species were affected, and the number of animals that died are critical pieces of information. Send specimens and the written history to the laboratory as soon as possible. Photographs can be helpful if they convey specific information, such as environmental conditions during a die-off and the appearance of sick wildlife or gross lesions (Figs. 1.1, 1.2).

Photos by Ronald Windingstad

Figure 1.1 *Examples of poor and good photography to record environmental conditions associated with wildlife disease problems.* ***(A)*** *Landscape photo displays topography and presence of a power line that may or may not be involved with the mortality event. Neither of the major factors involved with this event can be clearly seen.* ***(B)*** *Closeup photograph clearly shows both the species involved and the peanuts that proved to be contaminated with the mycotoxins that were the source of the problem.* ***(C)*** *Closeup photograph of sick bird clearly illustrates clinical signs of wing and neck droop; and the snow indicates the season.*

Photos by Milton Friend

Figure 1.2 *The observer may use photography to illustrate field observations associated with wildlife morbidity and mortality.* ***(A)*** *For example, when sick birds are left undisturbed or approached quietly, they often remain motionless along the water's edge with their heads hanging down. When startled, these birds may attempt to escape by propelling themselves with their wings across water* ***(B)*** *or land* ***(C)*** *but are unable to fly.* ***(D)*** *This bird has lost the use of its legs, a common occurrence with avian botulism and certain toxins such as organophosphorus or carbamate compounds.*

The following basic information is helpful for diagnosing the cause and assessing the severity of a wildlife health problem. Waterfowl are used as an illustrative example.

Environmental Factors

Determine if the start of mortality coincided with any unusual event. Environmental changes such as storms, precipitation, and abrupt temperature changes are potential sources of stress that can contribute to disease outbreaks. A food shortage may degrade the condition of birds and increase their susceptibility to disease. Water-level changes in an area may concentrate or disperse birds, alter the accessibility of toxins in food or water, or cause an invertebrate die-off that could lead to an avian botulism outbreak. Attempt to determine whether or not biting insect populations have increased or if such insects are present, because some insects are carriers of blood-borne infections in waterfowl.

The quality of the water used as a source for an impoundment may contribute to disease or mortality; for example, poor water quality may contribute to avian botulism or may be a primary cause of mortality if water contamination by toxic materials and substances such as oil, which can affect the integrity of feathers, is severe. Record recent pesticide applications and other habitat or crop management practices as well as previous disease problems in the area.

Estimating Disease Onset

When estimating the onset of disease, consider: (1) the earliest date when on-site activities could have resulted in the detection of sick or dead birds, if they were present, and the actual date when diseased birds were first seen, and (2) the proportion of fresh carcasses compared with the number of scavenged and decomposed carcasses. The abundance and types of scavengers and predators can be used to predict how long carcasses remain in the area. Other useful information about the onset of mortality can be gained from noting any differences in plumage, including stage of molt, if present, between live and dead birds. Size differences between live and dead nestlings and fledglings may also provide useful information for comparison with known growth rates. Also, air, water, and soil temperatures will affect the speed of decomposition and they should be considered in assessing how long birds have been dead. Include these observations in the history.

Species Affected

Much can be learned by knowing what species are dying. Those species present but unaffected are especially important to note, because some diseases infect a narrow host range and others infect a wide variety of species. For example, duck plague affects only ducks, geese, and swans, but avian cholera affects many additional species of water birds as well. Species with similar feeding habits may be dying as a result of exposure to toxins, while birds with different food requirements remain unaffected.

Age

Some disease agents may kill young birds but leave adults unaffected because of age-related disease resistance; other diseases kill birds of all ages, although young or old birds may be more susceptible because of additional stress placed on these age groups. When toxins are involved, differences in food habits may result in exposure of young birds, but not of adult birds, or vice versa.

Sex

Sex differences in mortality may be apparent in colonial nesters where females are incubating eggs, or in other situations where the sexes are segregated.

Number Sick/Number Dead

The longer a disease takes to kill, the more likely it is that significant numbers of sick birds will be found. For example, more sick birds will probably be observed during an avian botulism die-off than during an outbreak of a more acute disease such as avian cholera.

Clinical Signs

When observing sick birds, describe the clinical signs in as much detail as possible. Include any abnormal physical features and describe unusual behaviors, such as a sick bird's response to being approached. Photographs (Fig. 1.2) of various behaviors or conditions associated with a disease can be especially useful and should be included with the history.

Population at Risk

Try to determine what species, and in what numbers, are in the vicinity of the die-off. This information can provide clues about the transmissibility of disease, and it may be useful during control efforts.

Population Movement

Record recent changes in the number of birds in the area, as well as the species present. In particular note the presence of endangered species. If bird numbers have increased, try to determine where they came from; if bird numbers have decreased, attempt to determine where they have gone. This can often be accomplished when population movements are being monitored for census, hunting forecasts, and other purposes. State, Federal, and private refuge personnel and other natural resources managers are good primary sources of information.

Specific Features of Problem Areas

Describe the location of a die-off so that a relatively specific area can be identified on a road map. Also include any available precise location data, such as global positioning information or data that will facilitate entering of specific locations into geographical information system databases. Describe the problem area in terms that are sufficiently graphic so that someone with no knowledge of it can visualize its major characteristics, such as topography, soil, vegetation, climate, water conditions, and animal and human use.

Example description of die-off location

The problem area is a 10-acre freshwater pond located in Teno County, North Carolina, 1/2 mile east of County KV, 5 miles north of Highway 43. The pond has an average water depth of 6–12 feet and a sandy substrate. Vegetation around the pond border is bullbrush and reed canary grass. The surrounding uplands are essentially flat for one-half mile in all directions and lie fallow, covered with grasses and some shrubs. The area is coastal with enough relief to prevent saltwater intrusion into the pond even during major storms. Weather for the past 2 weeks has been pleasant and there has been no precipitation. Daytime temperatures are currently in the mid-80s (°F) and evening temperatures in the 70s. This is an isolated body of freshwater with good clarity, and sustains several hundred waterfowl, gulls, and small numbers of wading birds and shorebirds, and healthy warm water fish and amphibian populations. Cattle graze the adjacent area. There are no residential or industrial buildings within 1 mile of the site. Human visitation is frequent for bird watching, fishing, and hiking. Companion animals such as dogs are allowed on the area.

Identify where sick and dead birds are found. Especially note the locations of groups of dead birds and any differences of habitat where dead and sick birds are found. Birds found in agricultural fields may be dying of pesticide exposure, birds with more chronic toxicoses usually seek dense cover, and birds dying of acute diseases may be found in a variety of situations. Check any relation between specific bird use of the area and the location of affected birds, such as roost sites, loafing areas, and feeding sites.

If followup investigations are conducted after specimens have been submitted, summarize the findings and observations of those investigations in a supplemental report to the original history. Maintain a copy of the new report in station

files, and provide a copy to the diagnostic laboratory where the specimens were sent. Both reports should contain the dates of the investigations, whether air or ground searches were performed, the number of investigators and the time spent on the investigation, the weather conditions, and the time of day when the site was investigated.

The insight provided by good specimen history data and by field observations is invaluable to disease specialists. This information enhances understanding of the ecology of disease, thereby serving as a basis for developing ways to prevent future die-offs or to reduce the magnitude of losses that might otherwise occur.

J. Christian Franson

Supplementary Reading

Wobeser, G.A., 1994, Investigation and management of disease in wild animals: New York, N.Y., Plenum Press, 265 p.

Chapter 2

Specimen Collection and Preservation

Specimens are used to provide supporting information leading to the diagnosis of a cause of disease or death. A specimen may be an intact carcass, tissues removed from carcasses, parasites, ingested food, feces, or environmental samples. The specimen should be as fresh and undamaged as possible.

Choosing a Specimen

An entire, fresh carcass is the best specimen to submit to the laboratory for diagnosis. This allows the diagnostician to assess all of the organ systems and to use appropriate organs for different diagnostic tests. Obtain the best specimens possible for necropsy; decomposed or scavenged carcasses are usually of limited diagnostic value. A combination of sick animals, animals that were euthanized after clinical signs were observed and recorded, and some of the freshest available carcasses compose an ideal specimen collection. The method of euthanasia should not compromise the diagnostic value of the specimen (see Chapter 5, Euthanasia). More than one disease may be affecting the population simultaneously, and the chances of detecting multiple diseases will be maximized if both sick and dead animals are collected. Specimens submitted should be representative of the species involved. If more than one species is affected, collect several specimens of each species; try to obtain a minimum of five specimens per species.

Tissue Collection

The primary consideration when collecting carcasses or tissues for diagnosis should be personal safety. Some wildlife diseases are transmissible to humans, and every carcass should be treated as a potential health hazard. Wear disposable rubber or plastic gloves, coveralls, and rubber boots. If gloves are not available, inverted plastic bags may be used (Fig. 2.1). Before leaving an area where carcasses are being collected, double-bag used gloves and coveralls, and disinfect boots and the outside of plastic bags with a commercial disinfectant or a 5 percent solution of household chlorine bleach. Also, double-bag specimens in plastic before removing them from the area. These precautions will help protect the people in the field and minimize transmission of disease to unaffected wildlife populations.

If it is impossible to submit an entire carcass for diagnosis, appropriate organs must be removed from specimens. If possible, do not dissect carcasses in the field without first consulting disease specialists about methods of dissecting and preserving tissues or parasites or both. Assistance can be obtained from a variety of sources (Appendix B). It is

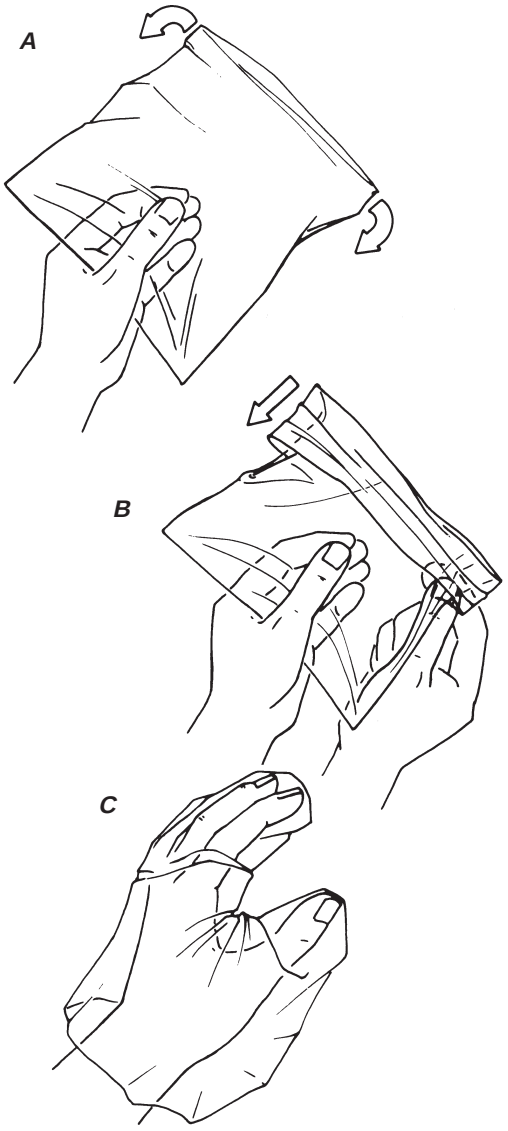


Figure 2.1 *Use a plastic bag to protect hands from direct contact with animal tissues during the collection of specimens if plastic or other waterproof gloves are not available.* ***(A)*** *Grasp bag at the bottom and* ***(B)*** *with other hand pull open end down over hand holding bag* ***(C)****. Repeat for the "unbagged" hand. Reversing this process when handling small specimens will automatically place specimens in the bag, which then need only be sealed and put into a second bag for packaging and shipment.*

best to become familiar with these sources and their ability to provide specific types of assistance before an emergency arises. The basic supplies and equipment that should be included in a field kit for specimen collection will vary with the species being sampled and the types of analyses that will be conducted. Keep a small kit packed in a day pack for ready use (Fig. 2.2). Sources of supplies used for collecting, preserving, labeling, and shipping specimens are listed in Appendix C.

Whirl-Pak® bags are very effective containers for tissue specimens. These bags have a sterile interior, are easy to carry in the field, and can be used to hold a variety of samples (Fig. 2.3). Specimen identification should be written directly on the bag with an indelible marker.

If lesions are noted, collect separate tissue samples for microscopic examination, microbiology, toxicology, and other analyses. With a sharp knife or scalpel cut a thin (l/8–1/4 inch, 3–6 millimeter) section of tissue that includes all or portions of the lesion and adjacent apparently healthy tissue (Fig. 2.4). Take care not to crush tissue in or around the lesion. Place the tissue sample in a volume of l0 percent buffered formalin solution equal to at least 10 times the tissue volume to ensure adequate preservation. Formalin is classified as hazardous; take appropriate measures to prevent skin contact or vapor inhalation. Jars, such as pint or quart canning jars, are convenient containers for preservation of tissues, but wide-mouth plastic bottles (Fig. 2.5) eliminate the potential breakage problems. After 2 or 3 days in 10 percent formalin, tissues can be transferred to Whirl-Pak® bags that contain enough formalin to keep the tissues wet. Write the specimen identification with indelible marker or pencil on a piece of index card, place the card inside the bag, and write the information directly on the bag with indelible marker. Pack the bags for shipping so as to prevent tissues from being crushed. Check with the courier regarding current requirements or restrictions for shipment of formalin.

If it is necessary to collect a blood sample from a live bird (if, for example, botulism is suspected), and syringes and needles are not available, sever the bird's head from its neck and collect the blood in a wide-mouth plastic jar.

Photographing external and internal lesions provides a record of the color, location, and appearance of lesions when appropriate camera equipment is available. Use a macro lens, high speed film, and a fast shutter speed to achieve maximum depth of field and sharply focused photographs with a hand-held camera. Include in the photograph for scale a coin or another readily recognized indicator of actual size. Explain on the history form submitted with the specimens what photographs were taken.

Photo by James Runningen

Figure 2.2 *A basic necropsy kit that can be packed into a small day pack. Clockwise, from top of photo: Data recording: field notebook, tags, pencils, markers. Protective apparel: rubber gloves, disposable shoe covers and coveralls, mask. Necropsy equipment: disinfectant for cleaning instruments, scrub brush, heavy shears, forceps, scissors, scalpel handle and blades. Measuring equipment: hanging scale and ruler. Sampling materials: microscope slides, syringes and needles, swabs, blood tubes, aluminum foil, Whirl Pak® bags, plastic bags, wide mouth plastic jars. Preservatives: ethanol for parasites, formalin for tissue samples.*

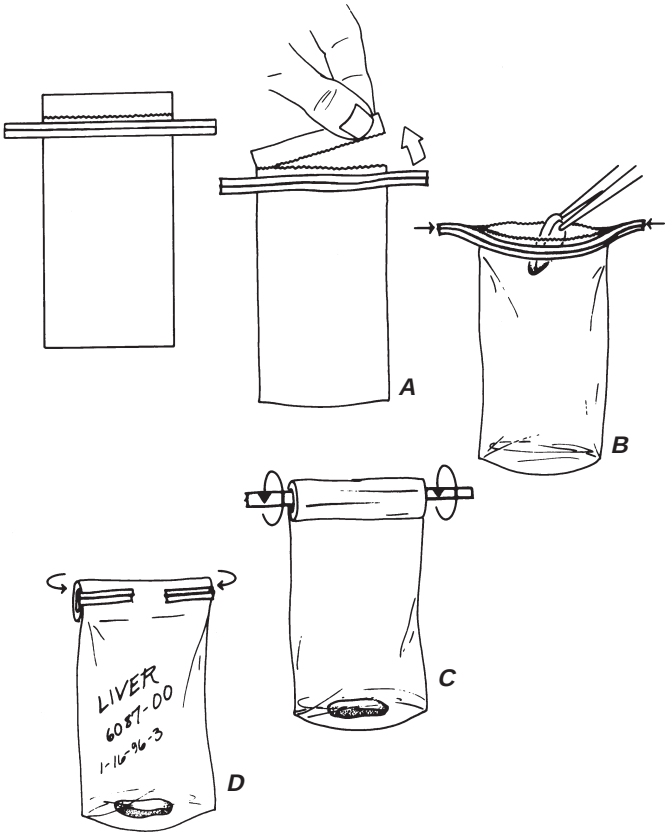


Figure 2.3 *Using Whirl-Pak® bag for specimen collection.* ***(A)*** *Remove top at perforation.* ***(B)*** *Open bag by simultaneously pushing the protruding wire-reinforced tabs toward the center to insert the specimen and any appropriate preservative.* ***(C)*** *Close bag by pulling on tabs and then twirling bag while holding tabs.* ***(D)*** *Secure the closure by folding tabs around bags and label bag with type of specimen, date, and any identifying numbers.*

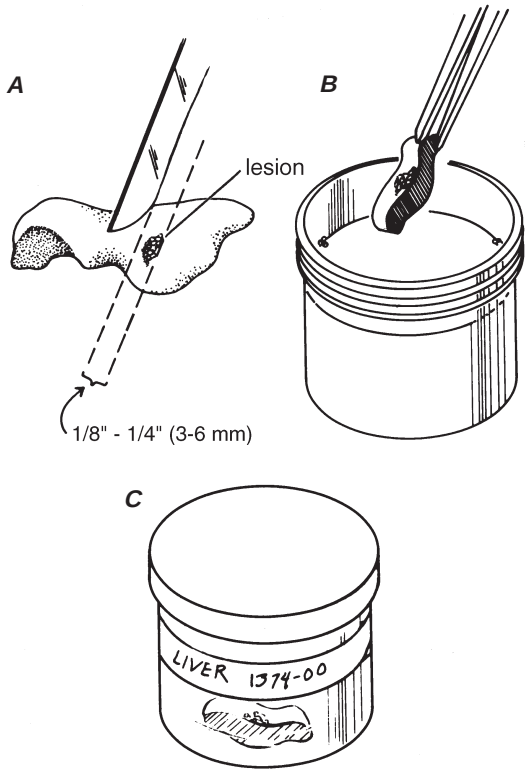


Figure 2.4 *Tissue sample collection for microscopic examination.* ***(A)*** *Tissue sample should include lesion, such as spots in liver, plus some apparently healthy tissue. The sample must be no thicker than 1/4 inch to ensure adequate chemical fixation by preservative. Use as sharp an instrument as possible (scalpel, knife, razor) for a clean cut.* ***(B)*** *Place tissue sample into container of 10 percent buffered formalin or other suitable fixative or preservative. The volume of formalin in the container should be about 10 times the amount of tissue sample.* ***(C)*** *Complete the process by securing the lid and properly labeling the container.*

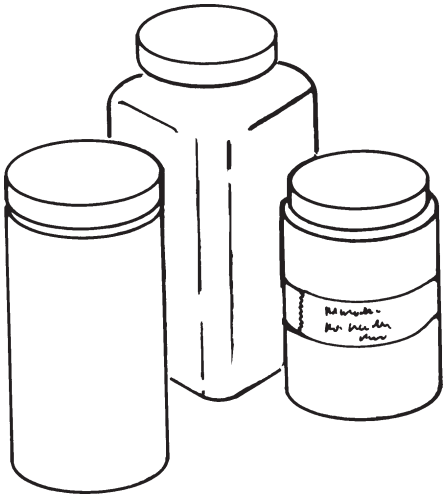


Figure 2.5 *Plastic bottles used for tissue specimens. Regardless of size or shape, specimen bottles should have a wide mouth and threaded caps for secure closure.*

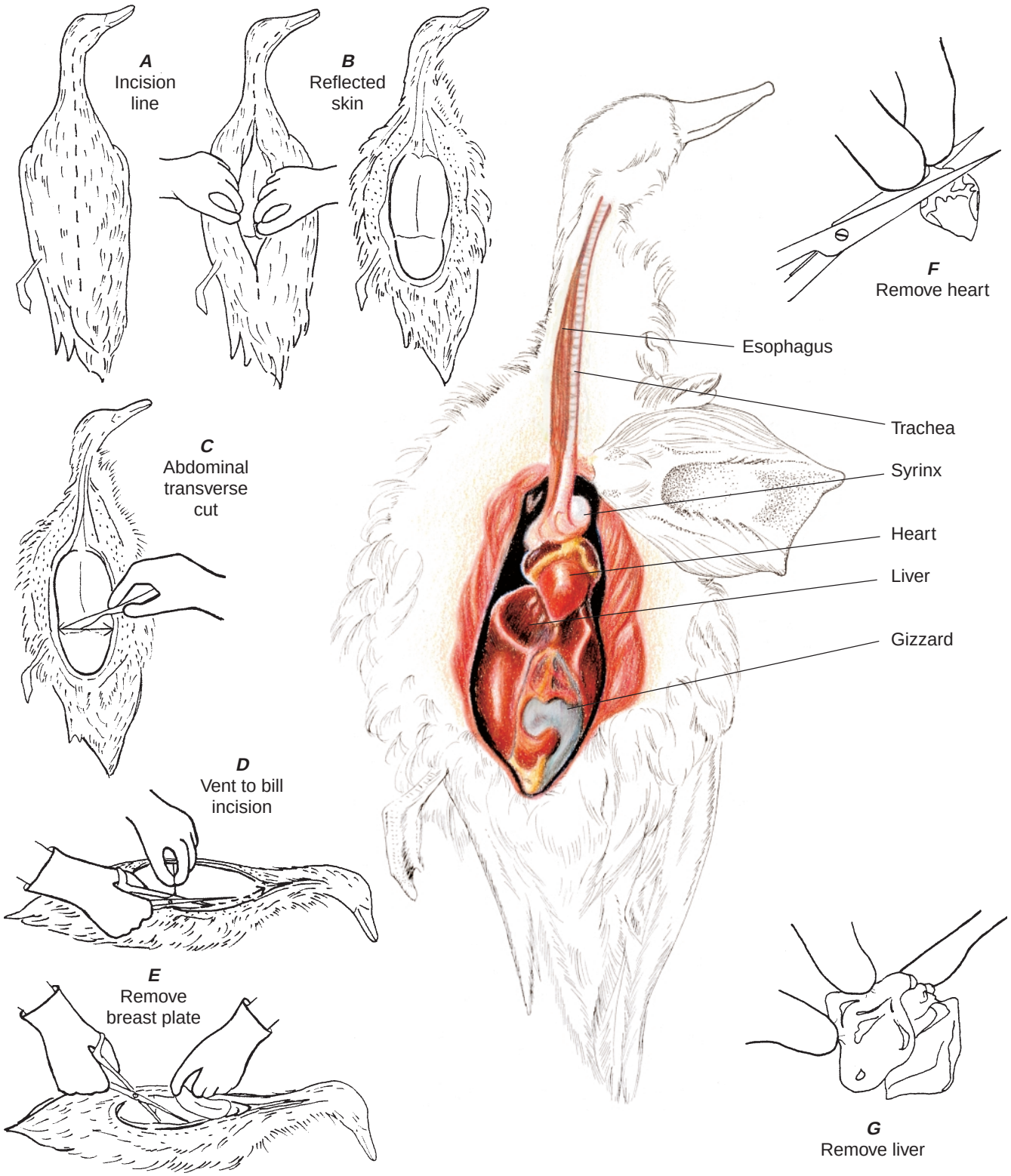
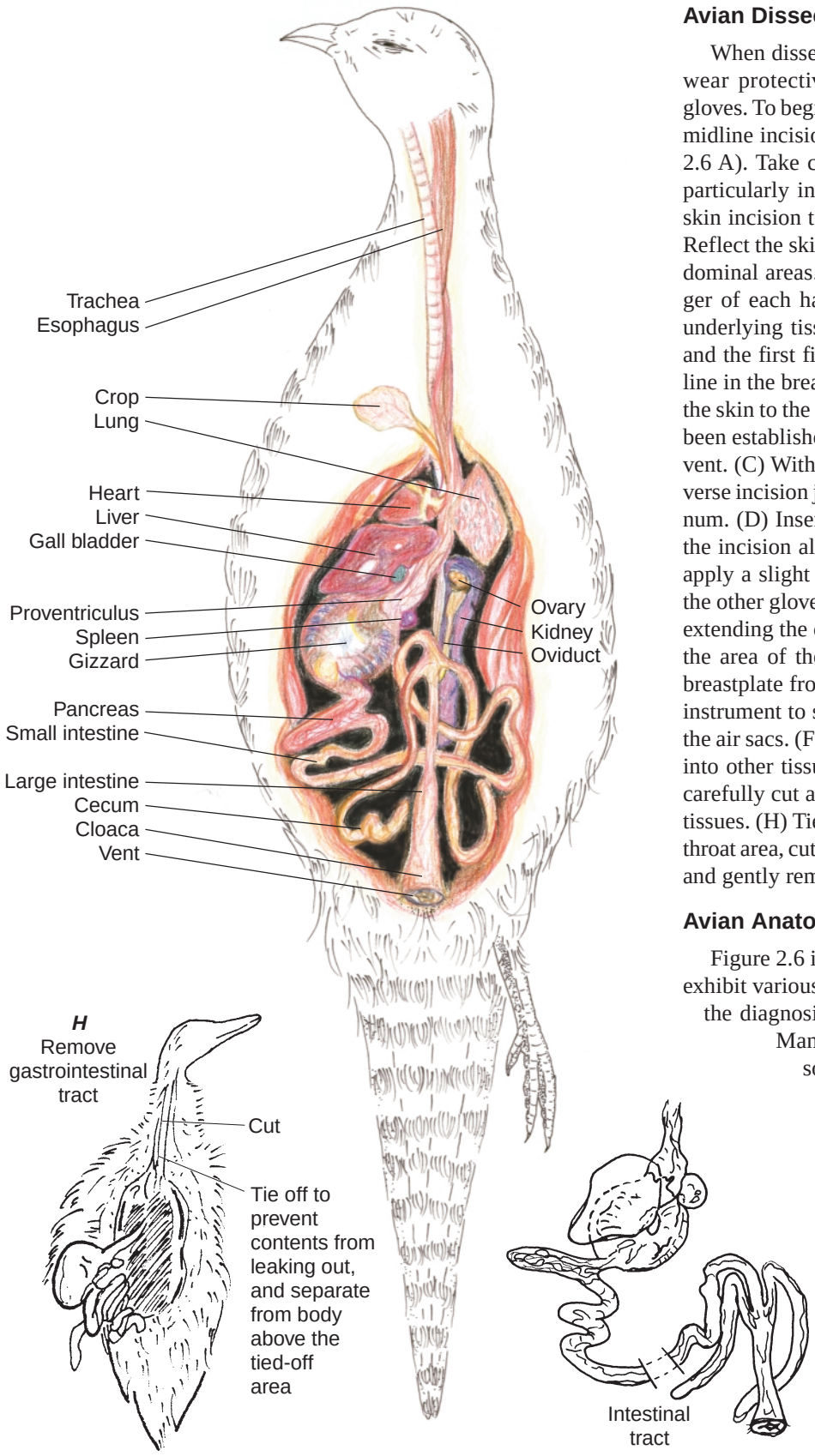


Figure 2.6 *Dissecting a duck carcass:* ***(A)*** *incision line;* ***(B)*** *reflect the skin to expose the underlying anatomy;* ***(C)*** *make a transverse abdominal cut below the breast muscle;* ***(D)*** *extend cut through the ribs and wishbone;* ***(E)*** *remove breast plate;* ***(F)*** *dissect out heart;* ***(G)*** *remove liver; and* ***(H)*** *tie off and remove the gastrointestinal tract.*



Avian Dissection

When dissecting a bird, it is always advisable to wear protective clothing, particularly disposable gloves. To begin, insert a scalpel or a knife to make a midline incision through the skin of the breast (Fig 2.6 A). Take care not to penetrate the body cavity, particularly in the abdominal region. Continue the skin incision to the vent and to the base of the bill. Reflect the skin away from the neck, breast, and abdominal areas. (B) Use the thumb and the first finger of each hand to reflect the skin to expose the underlying tissues. It is easiest to place the thumb and the first finger of each hand along the incision line in the breast area and then push and gently pull the skin to the side. When an opening in the skin has been established, work towards the bill and then the vent. (C) With a sharp blade, make a shallow transverse incision just below the breast muscles and sternum. (D) Insert the thumb of one gloved hand into the incision along the midpoint of the sternum and apply a slight pressure upwards. With a scissors in the other gloved hand, carefully cut through the ribs extending the cut on each side of the breast through the area of the wishbone. (E) Gently separate the breastplate from the carcass; use a scissors or other instrument to sever any connections and push aside the air sacs. (F) Dissect out the heart without cutting into other tissues. (G) Gently remove the liver and carefully cut away its area of connection with other tissues. (H) Tie off the gastrointestinal tract near the throat area, cut the esophagus above the tied-off area, and gently remove the entire gastrointestinal area.

Avian Anatomy

Figure 2.6 illustrates organs and tissues that may exhibit various lesions and that may be sampled for the diagnosis of disease agents described in this Manual. Species variation may result in some differences in the appearance and relative size of particular organs and tissues, but their location will be similar among species. Notable differences between the types of species illustrated are the small flat spleen in normal ducks and the larger oval spleen in pheasants. Also, pheasants have a crop and ducks do not; instead, the area just forward of the gizzard (the proventriculus) is more prominant in waterfowl.

Labeling Specimens

Proper labeling, maintaining label readability, and preventing label separation from specimens are as critical as proper specimen selection and preservation. The label should be as close to the specimen as possible; for example, a label should be attached to a carcass, attached to a tube of blood, or placed within the vial of preservative with a parasite. Double labeling, or placing a label on the outside of a plastic bag holding the specimen whenever practical, is worth the effort. The double labeling prevents confusion and potential

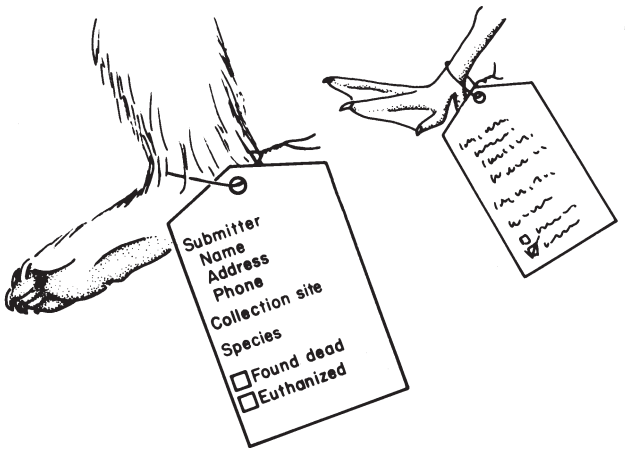


Figure 2.7 *Proper tagging of specimen. History of the specimen (see text for details) should be placed on back of tag.*

errors in specimen records at the diagnostic laboratory when specimens are received from multiple carcasses. Manila tags can be used, but take care to prevent their exposure to large amounts of fluids that may destroy the tag; tag destruction can be reduced by using tags with high rag content or even linen tags. Use soft lead pencil or waterproof ink on these tags; do not use ballpoint pen, nonpermanent ink, or hard lead pencil. The most durable tag is made of soft metal, such as copper or aluminum, and can be inscribed with ballpoint pen, pencil, or another instrument that leaves an impression on the tag.

Carcass

Identify each carcass with a tag fastened with wire to a leg (Fig. 2.7). If tags are not available, use a 3- by 5-inch card placed inside a plastic bag within the bag holding the carcass. Information on the tag should include the name, address, and telephone number of the submitter, collection site, species; whether the animal was found dead or was euthanized (indicate method); and a brief summary of any clinical signs. Place each tagged carcass in a separate plastic bag and seal the bag.

Tissues and Organs

When a specimen is in a plastic bottle, jar, or tube, wrap a piece of adhesive or masking tape entirely around the container and use an indelible marker to write on the tape. List the type of animal from which the sample was taken, the kind of tissue, and the date the sample was taken. When plastic bags are used as the first containers for tissues, they should be labeled with the same information directly on the bag. Do not insert tags inside containers with tissues and organs collected for microbiological or chemical analyses because the tag or the ink on it may contaminate the specimen. When chemically resistant tags are available, insert the tags into containers with preservatives such as formalin or alcohol.

Specimen Preservation

Chill or freeze all specimens, depending on how long it will take to ship to a diagnostic laboratory. Freezing reduces the diagnostic usefulness of carcasses and tissues, but if specimens must be held for 2 or more days, freezing the specimens as soon as possible after collecting them minimizes their decomposition. Formalin-fixed tissues should not be frozen. See Chapter 3, Specimen Shipment, for detailed instructions for packing and shipping specimens.

J. Christian Franson

(All illustrations in this chapter are by Randy Stothard Kampen, with the exception of Figure 2.6)

Supplementary Reading

Roffe, T.J., Friend, M., and Locke, L.N., 1994, Evaluation of causes of wildlife mortality, *in* Bookhout, T.A., ed., Research and Management Techniques for Wildlife and Habitats (5): Bethesda, Md., The Wildlife Society, p. 324–348.

Wobeser, G.A., 1997, Necropsy and sample preservation techniques, *in* Diseases of wild waterfowl (2nd ed): New York, N.Y., Plenum Press, p. 237–248.

Chapter 3

Specimen Shipment

Procedures for shipping specimens vary with different disease diagnostic laboratories. Therefore, it is important to contact the receiving laboratory and obtain specific shipping instructions. This will facilitate processing of specimens when they reach the laboratory and assure that the quality of specimens is not compromised. Time spent on field investigation, specimen collection, and obtaining an adequate history will be of little value if specimens become contaminated, decomposed, or otherwise spoiled during shipping to the diagnostic laboratory.

There are five important considerations for proper specimen shipment: (l) prevent cross-contamination from specimen to specimen, (2) prevent decomposition of the specimen, (3) prevent leakage of fluids, (4) preserve individual specimen identity, and (5) properly label the package. Basic supplies needed for specimen shipment are shown in Fig. 3.l.

Preventing Breakage and Leakage

Isolate individual specimens from one another by enclosing them in separate packages such as plastic bags. Protect specimens from direct contact with any coolant used (e.g., wet ice or dry ice), and contain all materials within the package so that leakage to the outside of the shipment container is prevented if breakage occurs (e.g., blood tubes) or materials thaw (wet ice and frozen carcasses) due to transit delays.

Containing Specimens

Plastic bags should be strong enough to resist being punctured by materials contained within them and from contact with other containers within the package.

Styrofoam® coolers, shipped in cardboard boxes, are useful for their insulating and shock absorbing qualities. Styrofoam® at least 1–inch thick is preferred. When possible, select Styrofoam® coolers that have straight sides. Coolers that are wider at the top than at the bottom are more likely to break during transit than those with straight sides. Fill the space between the outside of the Styrofoam® cooler and the cardboard box with newspaper or other packing material to avoid cooler breakage (Fig. 3.2). If coolers are not available, cut sheets of Styrofoam® insulation to fit the inside of cardboard boxes.

The cardboard box protects the Styrofoam® cooler from being crushed during transit and serves as containment for the entire package (Fig. 3.3). The strength of the box should

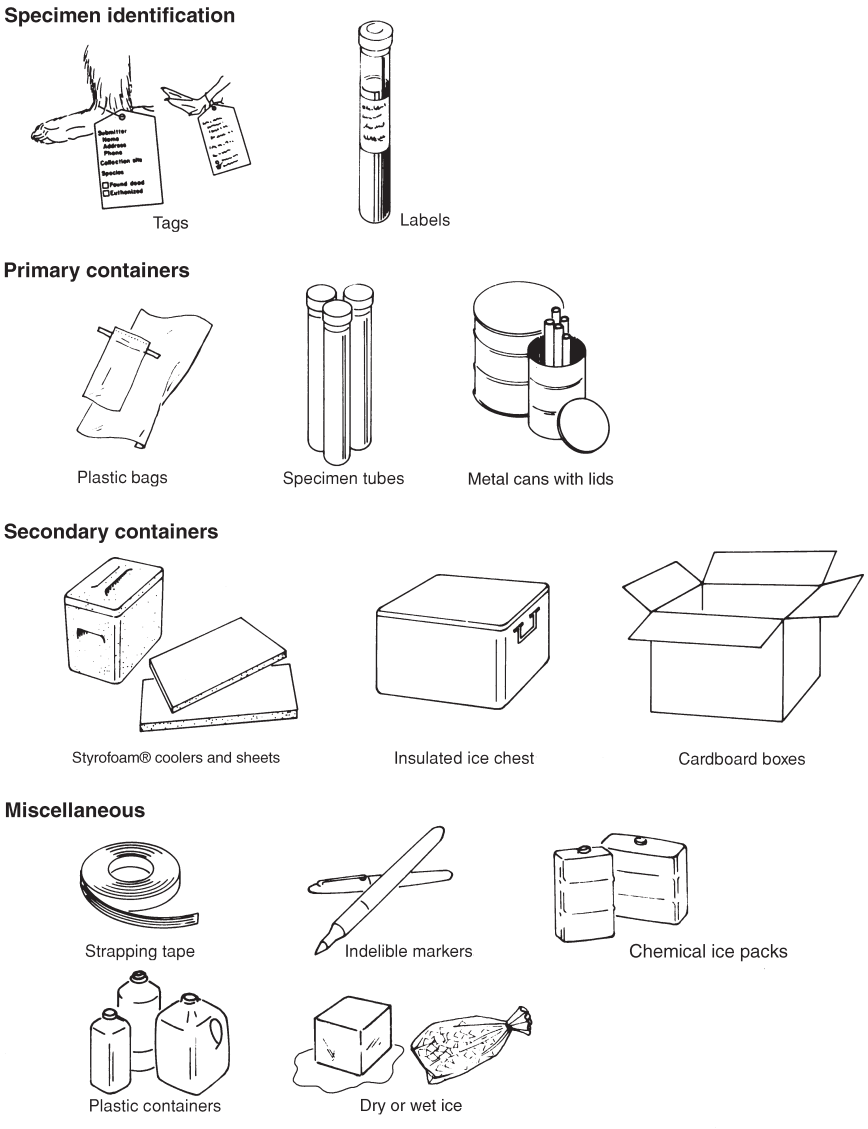


Figure 3.1 *Basic specimen shipment supplies.*

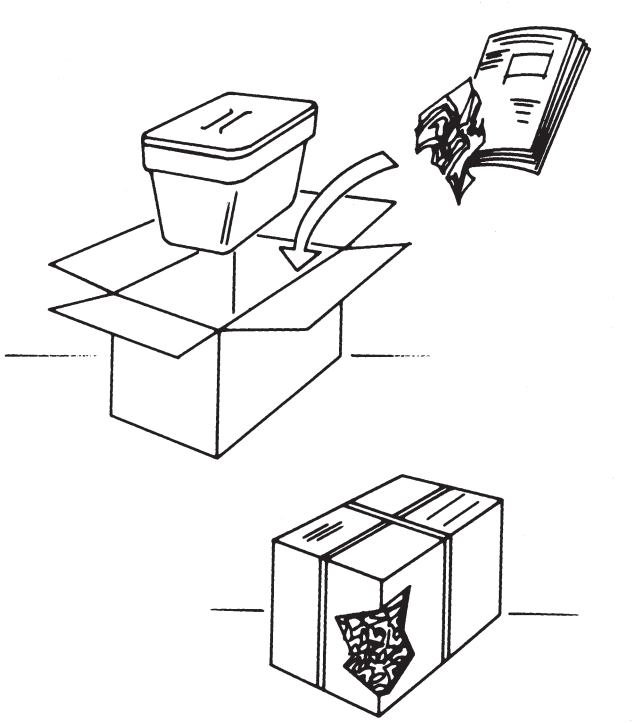
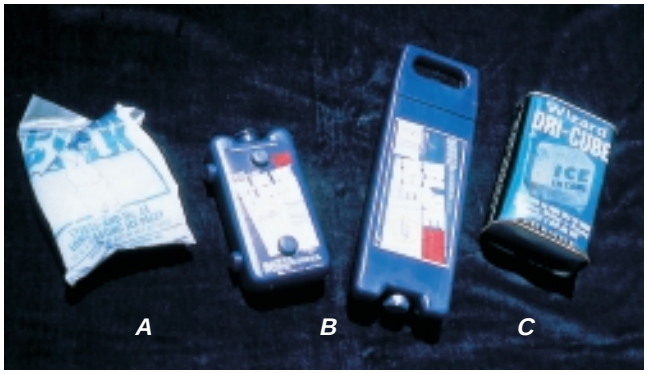


Figure 3.2 *Proper packing to prevent Styrofoam® coolers from becoming crushed during transit. Place the sealed Styrofoam® cooler in a sturdy cardboard box. Use crumpled newspaper or other packing material to fill all space between the cooler and the box.*

Figure 3.3 *This Styrofoam® cooler was not packaged in a cardboard box for shipping.*



Photos by James Runningen

Figure 3.4 *Chemical coolants are available in* ***(A)*** *soft plastic,* ***(B)*** *hard plastic, and* ***(C)*** *metal containers.*

be consistent with the weight of the package. Cardboard boxes are not needed when hard plastic or metal insulated chests are used for specimen shipment, but boxes can be used to protect those containers from damage and to provide a surface for attaching labels and addresses to the shipment.

Cooling and Refrigeration

Chemical ice packs (Fig. 3.4) are preferable to wet ice because their packaging prevents them from leaking when they thaw. Ice cubes or block ice may be used if leakage can be prevented. This can be accomplished most easily by filling plastic jugs such as milk, juice, and soda containers with water and freezing them. The lids of these containers should be taped closed to prevent them from being jarred open during transit.

Use dry ice to keep materials frozen, but do not use it to ship specimens that should remain chilled because it will freeze them. Also, the carbon dioxide given off by dry ice can destroy some disease agents; this is of concern when tissues, rather than whole carcasses, are being shipped. Shipment of dry ice, formalin, and alcohol is regulated and should be cleared with the carrier before shipping.

Preparing Specimens for Shipment to the National Wildlife Health Center (NWHC)

Other disease diagnostic laboratories may require minor variations in shipping procedures.

1. Call the NWHC (608-270-2400) to determine the optimal type and number of specimens for diagnostic procedures, how these specimens are best preserved during transit (whether they should be chilled or frozen), and when they should be shipped. In most cases, the NWHC requests that specimens be shipped the same day or within 24 hours.

2. Double-bag carcasses (Fig. 3.5) and place them in a Styrofoam® cooler lined with a plastic bag. When both frozen and fresh whole carcasses are shipped in the same container, the frozen carcasses can be used as a refrigerant to keep the fresh carcasses chilled. This can be accomplished by interspersing individually bagged frozen carcasses among the individually bagged fresh carcasses or by placing the fresh carcasses between two layers of frozen carcasses (Fig. 3.6). Blood tubes and other breakable containers of uniform size can be protected by packing them in a common plastic bag that is sealed within a metal can or a hard plastic container with a lid (Fig. 3.7). Pack any space around the specimen containers within the can (side and top) with paper or some other absorbent material to prevent jarring that could cause breakage and to collect fluids if tubes do break. Seal the can within a plastic bag before placing it in the Styrofoam® cooler.

3. When using chemical ice packs, intersperse them among specimens; place within the Styrofoam® container other types of coolants in locations that will provide maximum cooling for all contents or, if dry ice is used, will keep everything frozen (Fig. 3.8). Fill all empty space within the

Styrofoam® cooler with newspaper to prevent materials from moving during transit. The insulating properties of newspaper will also help maintain cool temperatures within the package, and its absorbent qualities will help prevent fluid leakage outside of the box or container.

4. Close the plastic bag lining the cooler and seal the lid with strapping tape (Fig. 3.9). Tape the specimen data sheet and history, contained in an envelope within a waterproof plastic bag, to the top of the cooler (Fig. 3.10A).

5. Enclose the Styrofoam® cooler in a cardboard box and secure the contents with strapping tape (Fig. 3.10B).

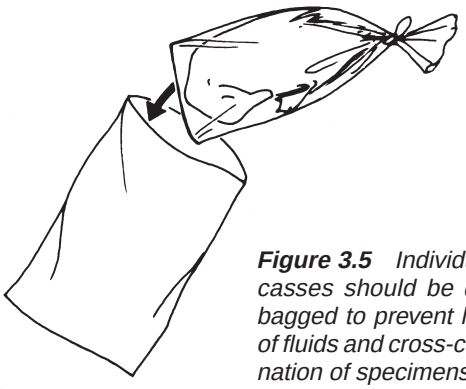


Figure 3.5 *Individual carcasses should be double-bagged to prevent leakage of fluids and cross-contamination of specimens.*

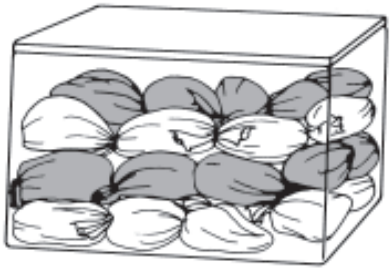


Figure 3.6 *Frozen carcasses (white bags) can be used to keep fresh specimens (dark bags) chilled during short transit times of 24 hours or less. Fill the space between the carcasses and the top of the container with newspaper to provide additional insulation to maintain the cold temperature.*

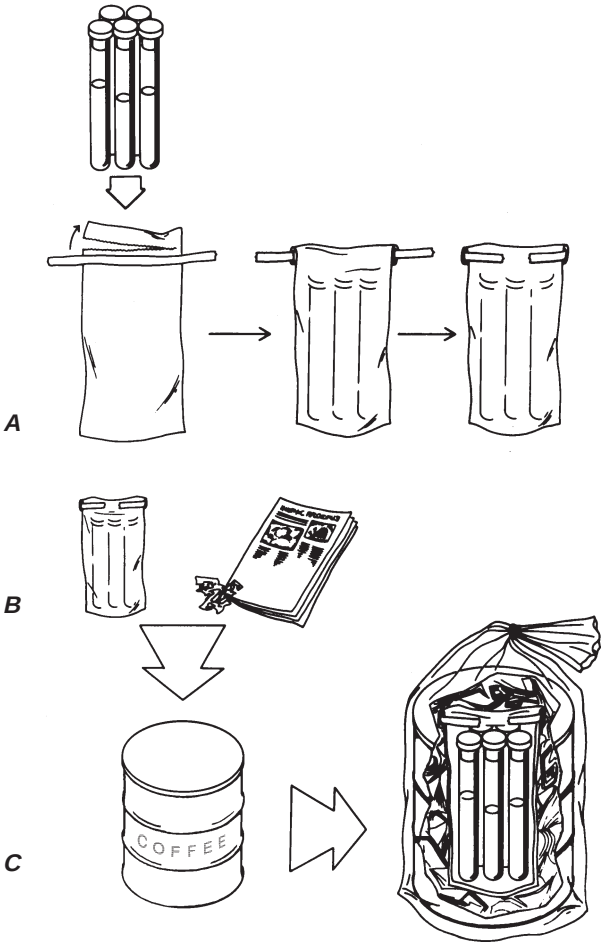


Figure 3.7 *Packing sequence for blood tubes.* ***(A)*** *Pack blood tubes within Whirl-Pak® or other plastic bag;* ***(B)*** *place bag in metal can or hard plastic container and pack with crumpled newspaper or other absorbent, soft, space-filling material; and* ***(C)*** *enclose the can in a plastic bag, then seal the bag.*

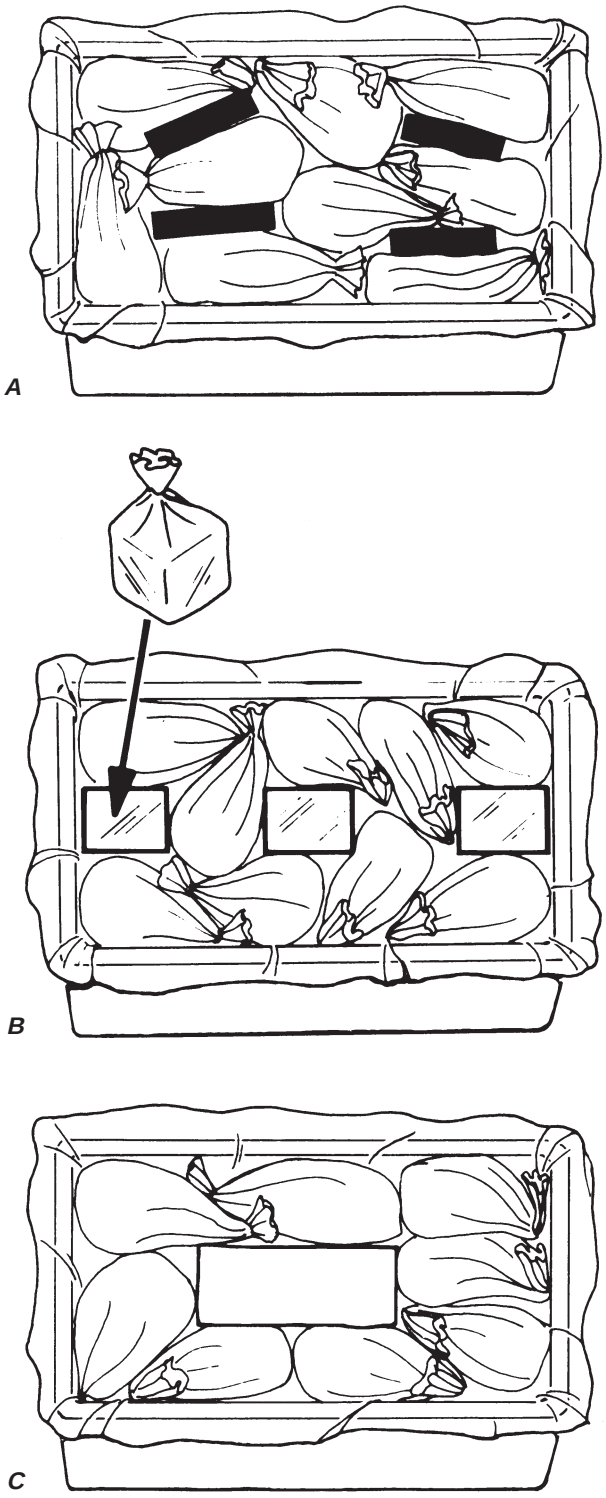


Figure 3.8 *Packing specimens for shipment when* ***(A)*** *ice packs* ***(B)*** *wet ice, and* ***(C)*** *dry ice are used as coolants. Note that the shipping container is always lined with a large plastic bag.*

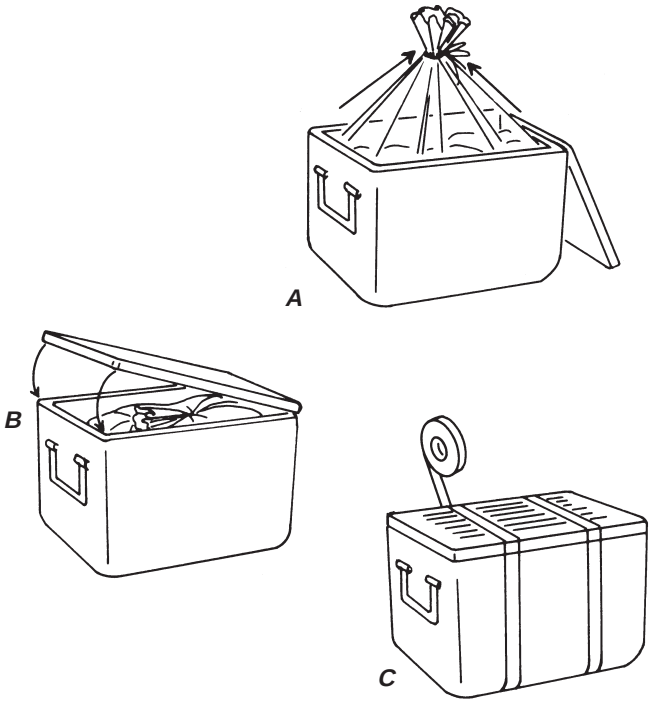


Figure 3.9 *Closing a specimen container.* ***(A)*** *Secure the large plastic bag containing the specimens by tying the top;* ***(B)*** *close the container lid and* ***(C)*** *secure the container with several bands of strapping tape.*

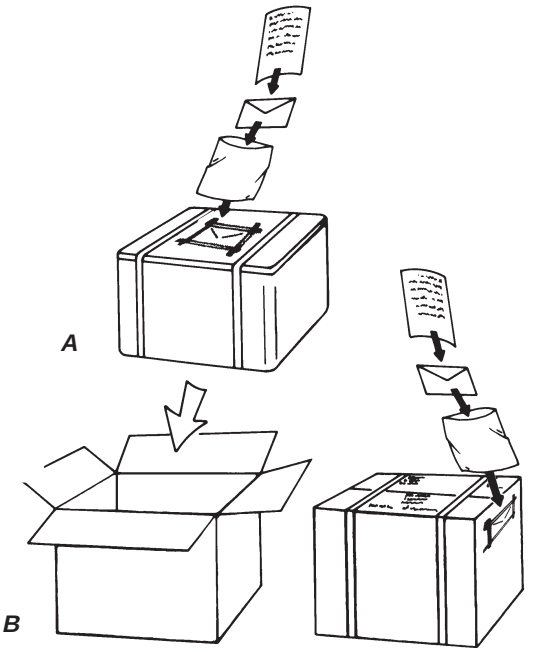


Figure 3.10 *Completing the packaging process.* ***(A)*** *Tape specimen data sheet and history, contained in an envelope within a waterproof plastic bag, to top of cooler.* ***(B)*** *Place cooler in cardboard box, secure box with several bands of strapping tape, and secure another copy of the specimen data sheet to the outside of the box. If the specimens were placed inside a Styrofoam® cooler, then use crumpled newspaper or other packing material to fill all spaces between the cooler and the box.*

Federal Shipping Regulations for Packaging and Labeling

Your packaging and labeling of specimens must conform to the following regulations.

The Code of Federal Regulations (CFR) states under 50 CFR Part 14 of Fish and Wildlife Regulations that containers with wildlife specimens must bear the name and address of the shipper and consignee, and a list of the species and numbers of each species must be conspicuously marked on the outside of the container. You may instead conspicuously mark the outside of each package or container with the word "wildlife" or the common names of the species contained within the package. Secure an invoice or packing list that includes the name and address of the consignee and shipper and that accurately states the number of each species contained in the shipment to the outside of one container in the shipment.

In addition to Fish and Wildlife Service regulations, the interstate shipment of diagnostic specimens is subject to applicable packaging, labeling, and shipping requirements for disease-causing etiologic agents (42 CFR Part 72). These regulations do not require you to identify diagnostic specimens as etiologic agents when the disease agent is not known or is only suspected. However, all specimen packages sent to the NWHC should be prominently labeled with the words "DIAGNOSTIC SPECIMENS." You can meet packaging requirements under 42 CFR Part 72 by following recommendations 2 through 5 above for enclosing specimens within two containers before enclosing them within the package.

Hazardous Materials Regulations of the Department of Transportation apply whenever dry ice is contained within the shipping container (49 CFR Part 172, 173, 175). Always call the carrier ahead of time for the current shipping and package labeling requirements. At the time of this writing, the following must be clearly visible on containers with dry ice: DRY ICE 9, UN1845, weight of dry ice (kilograms), a hazardous materials miscellaneous 9 sticker, and the complete addresses of the shipper and recipient. The dry ice labeling should go on the side of the container, so it is visible if something is stacked on top of it. Always include the words "DIAGNOSTIC SPECIMENS (WILDLIFE)" on the container. A properly labeled container is illustrated in Fig. 3.11. Label containers with permanent markers, if possible.

Commercial Carriers

Specimens should be shipped by carriers that can guarantee 24-hour delivery to the location of the diagnostic laboratory. For many locations, commercial delivery services will pick up packages at the point of origin. When shipping arrangements have been made, contact the NWHC again and provide the airbill number and estimated time of arrival. This information is needed to allow prompt tracing of shipments that may not arrive on schedule and to schedule work at the laboratory.

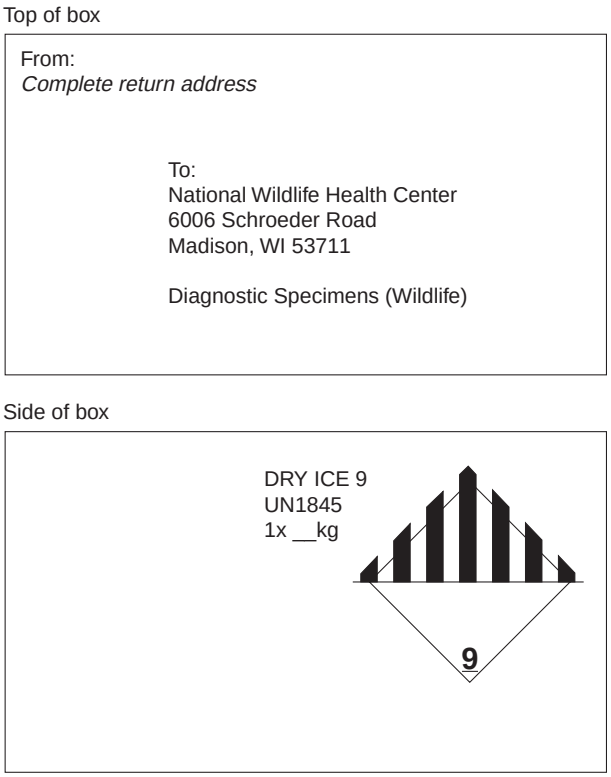


Figure 3.11 *Proper package labeling.*

J. Christian Franson

(All illustrations in this chapter are by Randy Stothard Kampen, with the exception of Figure 3.11)

Supplementary Reading

Code of Federal Regulations. Title 42; Part 72
Code of Federal Regulations. Title 49; Parts 172, 173, 175.
Code of Federal Regulations. Title 50; Part 14.

Chapter 4

Disease Control Operations

Individual disease outbreaks have killed many thousands of animals on numerous occasions. Tens of thousands of migratory birds have died in single die-offs with as many as 1,000 birds succumbing in 1 day. The ability to successfully combat such explosive situations is highly dependent on the readiness of field personnel to deal with them. Because many disease agents can spread through wildlife populations very quickly, advance preparation is essential for preventing infected animals from spreading disease to additional species and locations. Carefully thought-out disease contingency plans should be developed as practical working documents for field personnel and updated as necessary. Well-designed plans can prove invaluable in minimizing wildlife losses and the costs associated with disease control activities.

Although requirements for disease control operations vary and must be tailored to each situation, all disease contingency planning involves general concepts and basic biological information. This chapter, which is intended to be a practical guide, identifies the major activities and needs of disease control operations, and relates them to disease contingency planning.

Planning Activities

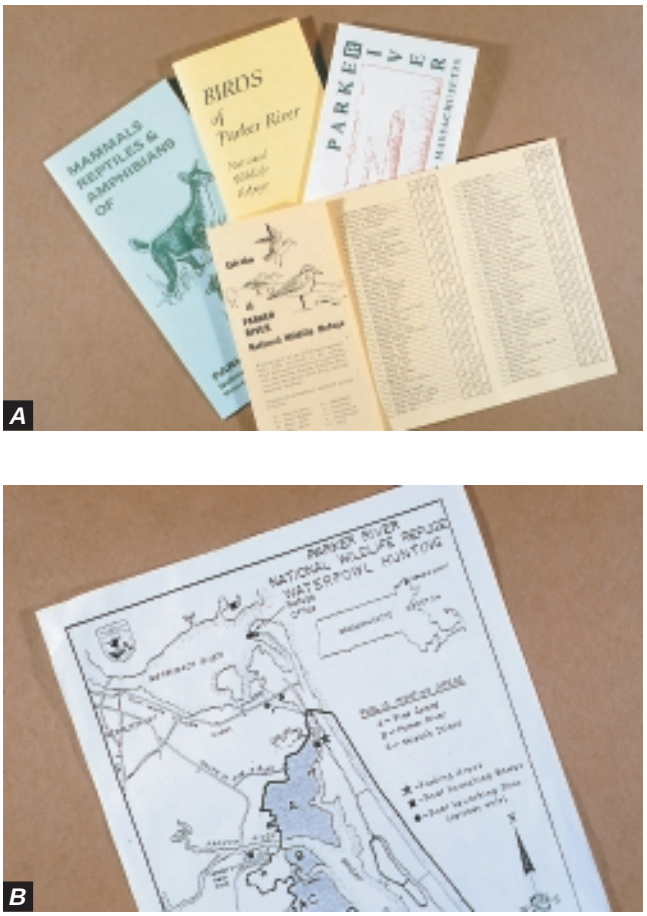
Identification of Needs

Effective planning for combating wildlife disease outbreaks requires an understanding of disease control operations and the basic needs such as personnel, equipment and supplies, permits, etc, that are associated with them (Tables 4.l and 4.2). This information is the basis of disease contingency planning (Table 4.3; Figs. 4.1 and 4.2).

Biological Data Records

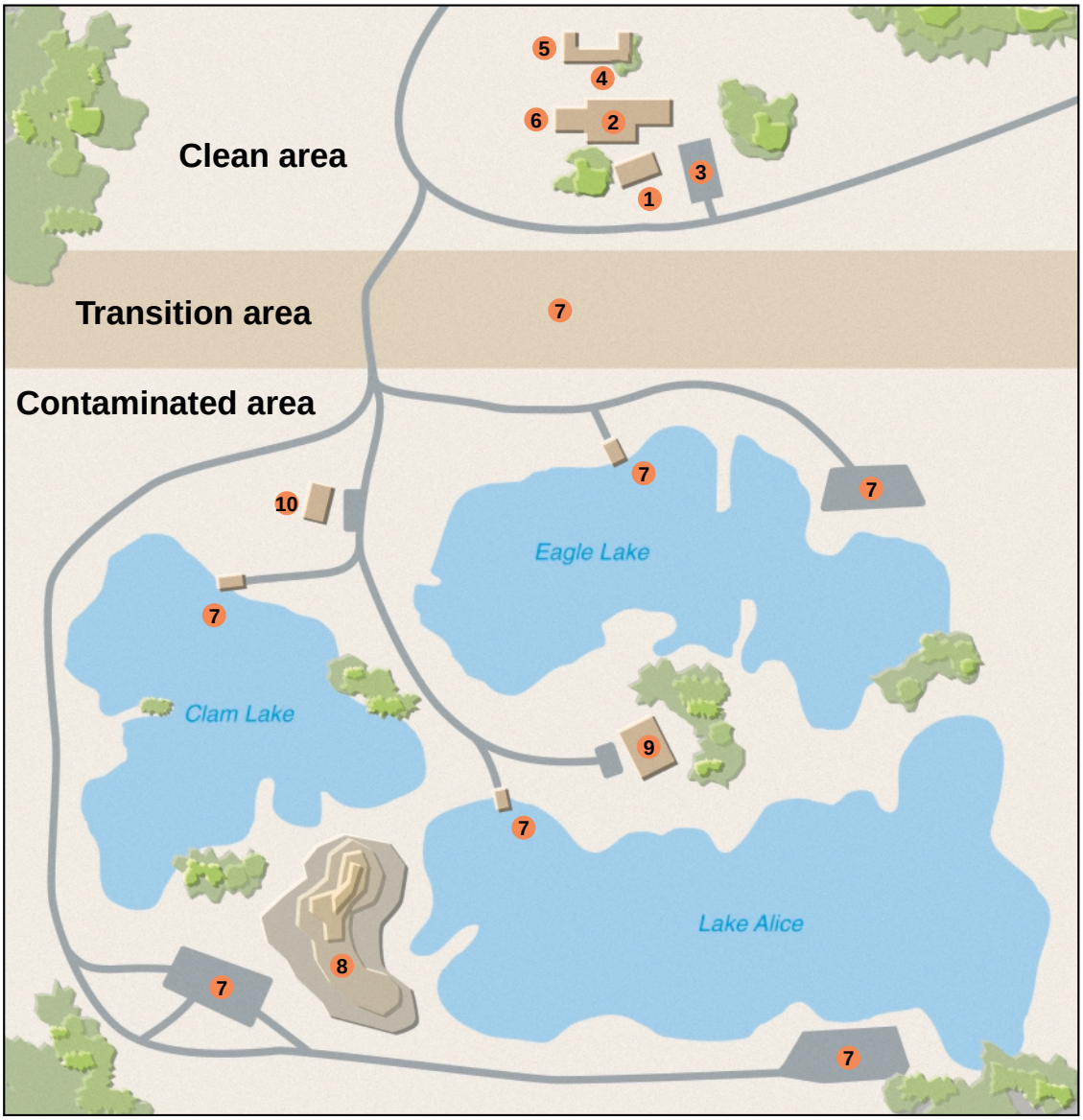
All disease outbreaks consist of three main components: a susceptible host population, a disease agent interface, and the environment in which the host and agent interact in a manner that results in disease. Disease control involves breaking the connections between these factors. Disease contingency plans expedite these efforts by providing basic information about the distribution and types of animal populations in the area, animal movement patterns, any history of disease problems on the area, and general environmental features. This information, along with facts gathered at the time of a disease outbreak, provides a profile for biological assessment and a basis for specific disease control actions.

Knowledge of the types of disease problems that have occurred in the area, their general locations, the month and year when they occurred, the species affected, and the general magnitude of losses is also of considerable value for planning a response to a disease outbreak. Incorporate a historical summary in tabular form in the contingency plan (Table 4.4). Animal population data are best represented by simple graphs and charts that convey general characteristics (Fig. 4.3); precise data are not needed. Generalized outline maps are useful for depicting concentration and feeding areas used by wildlife (Fig. 4.4) and major movement patterns (Fig. 4.5).



Photos by James Runningen

Figure 4.1 ***(A)*** *Station brochures, animal lists, and other public-use documents provide a wide variety of site-specific background information and should be included as part of the station's disease contingency plan.* ***(B)*** *Documents containing maps of the area indicating access points provide essential information.*



EXPLANATION

1. Command post and headquarters administrative area
2. Staff and press briefing room
3. Parking
4. Eating area and conference room
5. Staff rest area and visitors' center
6. Equipment and supply receipt—garage
7. Decontamination areas—boathouses, transition areas, parking lots
8. Carcass disposal site and observation hill
9. Animal holding—pole barn (has cement slab and electricity)
10. Laboratory investigations—shed (has cement slab, water, electricity)

Figure 4.2 *Existing work areas used for disease control operations on a wildlife management area.*

Response Activities

Response to wildlife die-offs will vary somewhat with the species but will always involve a set of common factors. Waterfowl die-offs are used to illustrate specific approaches to addressing these common factors. For large mammals, their size and weight pose additional needs regarding carcass transport and disposal.

Problem Identification

Early detection and rapid and accurate assessment of the causes of disease problems are essential to effective disease control operations. This is accomplished through surveillance of animal populations to detect sick and dead wildlife, and the prompt submission of specimens to qualified disease diagnostic facilities. The speed with which large numbers of animals can become exposed to disease agents and the differences in control activities required for different types of disease problems place a premium on both the speed and accuracy of diagnostic assessments. Once a disease problem has been identified, the following basic activities are carried out.

Carcass Removal: Protective Clothing and Supplies

Wildlife that have died from disease are often a primary source of the disease agent, and for most situations their carcasses need to be removed from the environment to prevent disease transmission to other animals through contact with or consumption of the carcass. Disease organisms released from tissues and body fluids as carcasses decompose also contaminate the environment. Some disease-causing viruses and bacteria can survive for several weeks or longer in pond water, mud, and soil.

Because carcass collection concentrates diseased material in a small area, it is essential that carcasses be handled so that they do not release infectious agents into the environment or jeopardize the health of personnel. Great care also needs to be taken to prevent mechanical movement of the disease agent from the problem area to other areas.

Personnel assigned to this task need to wear outer garments that provide a protective barrier against direct contact with disease organisms and that can be disinfected and removed before personnel leave the area. Typically, these include boots, coveralls or raingear, gloves, and a head covering (Fig. 4.6).

Use disposable coveralls and outer gloves when possible; the durability and cost of garments are considerations in decisions about whether or not disposable garments will be used. Personnel should remove coveralls and outer gloves before they leave the area, and the garments should be destroyed if they are disposable or they should be double-bagged before they are transported to a location where they can be thoroughly washed before they are reused. Dishwashing gloves, work gloves, and other types of rubber gloves are readily available at hardware and other retail stores, as are scrub brushes for cleaning (Fig. 4.7).

Carcass removal requires heavy-duty plastic bags or containers. Plastic body bags used by the military are excellent for containing wildlife carcasses. Plastic garbage cans lined with commercially available heavy-gauge leaf and litter plastic bags are also excellent containers for transporting carcasses. These containers are especially useful when personnel collect bird carcasses by boat (Fig. 4.8A), and for transporting carcasses in truck beds. Tie the bags shut and secure garbage can lids when transporting these containers to carcass disposal sites (Fig. 4.8B).

Depending on conditions, a variety of watercraft (Fig. 4.9) and all-terrain vehicles (Fig. 4.10) are useful for searching for carcasses and for transporting carcasses to collection and disposal sites. In some instances, the expense of helicopters may be warranted. Pickup trucks and other four-wheel vehicles are also indispensable under some field conditions.

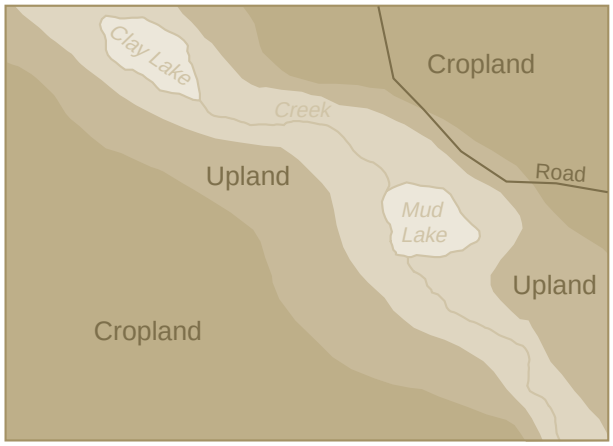
Dogs have been used extensively in wildlife management, and they are a valuable search tool when they are appropriately chosen and handled. Use dogs whenever possible to locate carcasses if there is no disease risk to them. Infectious diseases of wild North American birds do not pose a significant health threat to dogs. Determine disease risk on a case-by-case basis by consulting with wildlife disease specialists. Local retriever clubs or kennels may provide dogs.

The contingency plan should identify sources of various equipment, whether equipment can be borrowed or rented, and contact persons and their telephone numbers. Commonly used supplies and equipment needed to support disease control operations are summarized in Table 4.2.

Carcass Disposal

The primary goal of carcass disposal is to prevent spread of the disease agent to other animals through environmental contamination. Because personnel will handle concentrated amounts of infectious or highly toxic agents, this activity requires proper training and supervision. Incineration, burying, rendering, and composting are the four basic disposal methods.

Incineration is generally the preferred method for disposing of carcasses and contaminated materials associated with wildlife disease outbreaks. However, air-quality standards often preclude open burning, even for disease emergencies. Consider purchasing or constructing portable incinerators (Fig. 4.11) for areas with recurring disease problems if local regulations allow using such equipment. Portable garbage incinerators can sometimes be borrowed from State parks and other sources. If portable incinerators are not available, open burning with tires or other fuel or both can be used, depending on local air pollution standards. Carcasses may be burned either above or below ground (Fig. 4.12). It is important to keep the fire contained and to get sufficient air movement under the carcasses to maintain a hot fire and completely burn the carcasses. Wood, coal, fuel oil, napalm, and



April–August: Nesting birds and broods on Mud and Clay Lakes and adjacent uplands.

September–mid-October: Fall migrants using Mud and Clay Lakes.

Mid-October–January: During hunting season, birds concentrated on Clay Lake and adjacent marsh.

February–March: After hunting season, birds distributed between Mud and Clay Lakes.

A

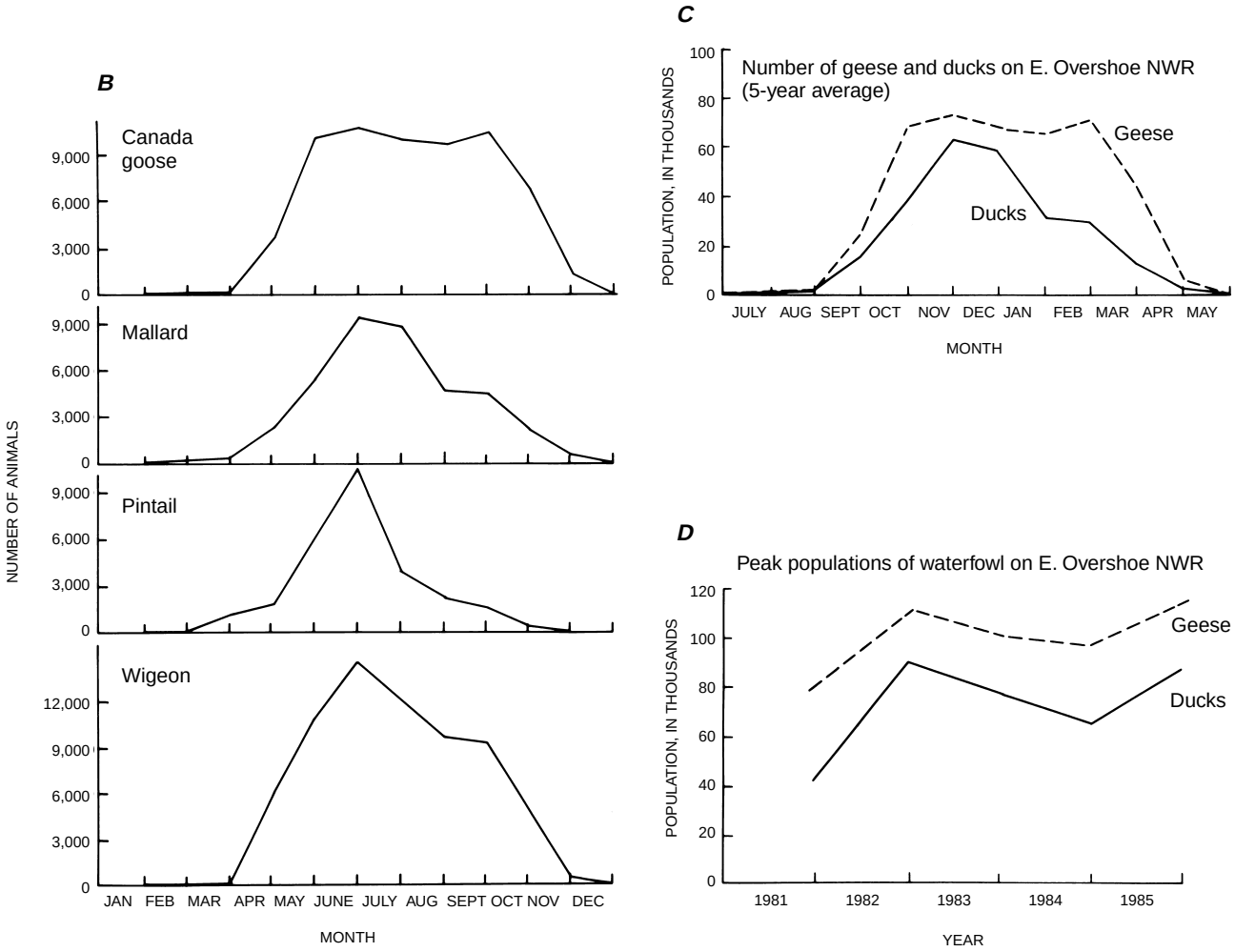
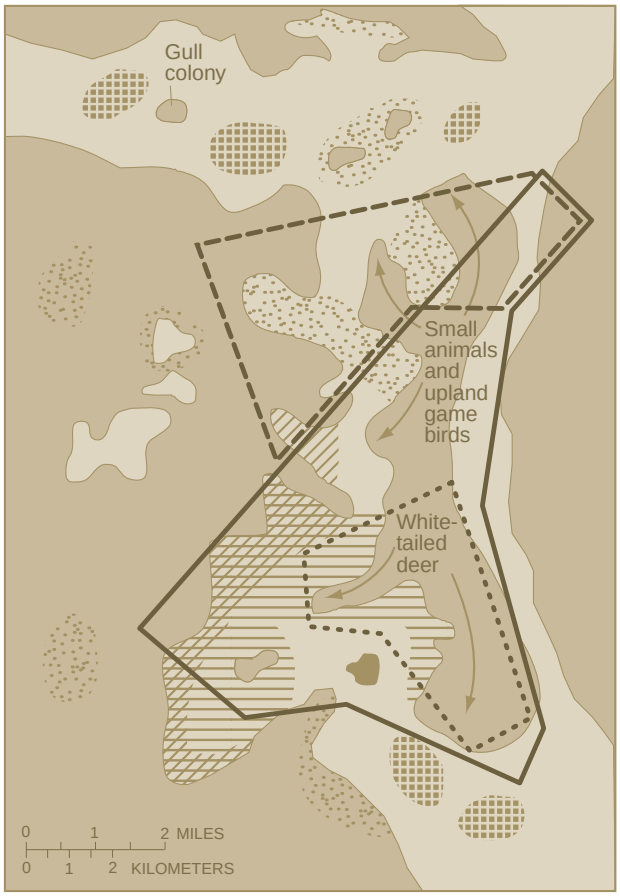


Figure 4.3 *Examples of how to present data on seasonal and annual wildlife use of a specific area.* ***(A)*** *General narrative format with map;* ***(B)*** *seasonal waterfowl populations by species, and total duck and goose use by* ***(C)*** *month and* ***(D)*** *year.*



EXPLANATION

Major use areas

- Shorebirds and wading birds
- Bald eagle wintering roost site

Waterfowl

- Loafing areas
- Roosting areas
- Feeding areas
- National Wildlife Refuge boundary
- National Wildlife Refuge Hunting Area
- State Game Management Area

Figure 4.4 *Example of an outline map showing concentration and feeding areas used by wildlife.*

EXPLANATION

Major movement patterns

- Puddle duck and bay diving duck feeding patterns
- Canada goose daily feeding flights

Major use areas

- White-tailed deer wintering area
- Spring migration diving duck staging areas

Figure 4.5 *Example of an outline map showing major movement patterns of species.*

Photo by William Bair, U.S. Fish and Wildlife Service

Figure 4.6 ***(A)*** *Protective clothing such as coveralls, boots, head coverings, and gloves should be warn during carcass cleanup activities.* ***(B)*** *Before leaving the area, boots should be decontaminated and outer clothing removed and bagged for transportation to a location where they can be washed before being re-used.*

Photo by Milton Friend

Photos by James Runningen

Figure 4.7 ***(A)*** *Examples of readily available disposable and reusable gloves for disease control operations. Dishwashing gloves, surgical gloves, rubber work gloves, and other types can be purchased at drug and hardware stores and medical and laboratory supply houses.* ***(B)*** *A wide variety of scrub brushes needed for decontaminating boots, equipment, and other surfaces are also readily available from local merchants.*

Photos by Milton Friend

Figure 4.8 ***(A)*** *Plastic barrel being used to transport carcasses from collection sites by airboat to disposal site. Note use of plastic bag to line barrel. The plastic bag containing carcasses can be secured, removed, and placed in a second plastic bag for further transportation if disposal site is not at the boat docking location, thereby allowing immediate reuse of the barrel. If the barrel containing carcasses is to be transported to some other location, the plastic bag should be tied closed and a cover placed on the barrel and secured.* ***(B)*** *Examples of improper transportation of carcasses to disposal site. Note untied bags, unbagged carcasses, wooden truck bed, and lack of tailgate. Carcasses and fluids contaminated with disease organisms could easily be released from the bags during transit. Fluids could contaminate the truck bed and leak to the ground through the cracks between the wooden boards. Wood absorbs fluids and is much more difficult to decontaminate than a nonporous surface. Also, carcasses could fall out of the truck because there is no tailgate.*

Photo by James Runningen

Photo by Milton Friend

Figure 4.9 *Different types of* ***(A)*** *motorized and* ***(B)*** *nonmotorized watercraft are useful for carcass collection. Note the use of plastic bags for containment of carcasses and further transportation to disposal sites.*

Photo by Ronald Windingstad

Photos by Milton Friend

Figure 4.10 *Selection of all-terrain vehicles should be matched to local conditions. All-terrain vehicles such as these three-wheel machines can* ***(A)*** *be equipped with small baskets to hold carcasses or live birds and* ***(B)*** *be used in water no more than 2-feet deep. Because of safety concerns, three-wheeled vehicles are not recommended.* ***(C)*** *Large tracked vehicles such as this equipment negotiate marshy terrain but are not amphibious. The major advantages of this equipment are the large capacity for carrying personnel, supplies, and equipment and excellent visibility afforded by the height of the vehicle.* ***(D)*** *Small amphibious vehicles such as this six-wheel machine are capable of transporting two persons and are more stable and versatile than three-wheel vehicles but are much slower on land surfaces.*

Photo by Milton Friend

Photo by Carl Batha, Wisconsin Department of Natural Resources

Photo by Milton Friend

Figure 4.11 *Examples of portable incinerators used for disease control operations.* ***(A)*** *Garbage incinerator borrowed from State park to dispose of carcasses during Lake Andes duck plague die-off.* ***(B and C)*** *Locally designed and constructed incinerators in use during disease control operations. All of these are fueled with propane gas.*

Photos by Milton Friend

Figure 4.12 *Examples of above-ground and in-trench methods for incineration of carcasses.* ***(A)*** *Portable grate the width of a pickup truck bed fashioned from metal pipes.* ***(B)*** *Simple grate suspended over pit into which carcass remains are placed for burial.* ***(C)*** *Major burning pit for large-scale operation—note surrounding area cleaned of vegetation for fire protection, the size and depth of pit, burning platform, rubber tires for fuel.—**Figure 4.12 is continued on p. 30.*

Figure 4.12—continued ***(D)*** *Intensity of heat generated by fire resulting in the bending of support pipes of the burning platform and metal grate.* ***(E)*** *Simple but sturdy above-ground structure of cinder blocks and steel grates elevated enough for fuel to be placed under the carcasses and for air to circulate upward.* ***(F and G)*** *Highly efficient above-ground burning platform constructed of a frame of used grader blades, wire mat platform, and* ***(H)*** *sheet metal heat deflector positioned at the rear of the platform.* ***(I)*** *Proper application of fuel oil (never use gasoline) for carcass incineration. Note that length of applicator prevents flashback or wind shift from endangering person applying fuel.*

Photos by Milton Friend

G

H

I

Photos by Milton Friend

other fuels have been successfully used. Never use gasoline because of the hazards involved. Incineration is facilitated by stacking or piling carcasses on the burning platform, soaking them with used oil or some other fuel, and waiting about 10 to 15 minutes before igniting them. The heat generated by large-scale carcass burning operations is intense enough to cause metal pipes to bend (Fig. 4.12D). Therefore, construct a sturdy carcass support surface so that it does not collapse into the fire.

During dry weather, burning carcasses in a pit surrounded by a vegetation-free area is more desirable than above-ground burning. In either situation, piling too many carcasses on the fire at once is a common mistake; burn carcasses one layer at a time (Fig. 4.13). When cinder blocks are used to support burning platforms, the length of the platform should be extended to keep the blocks out of direct heat or they will soon crumble.

When burning is not feasible or needed, burial is often a suitable alternative. Select burial sites carefully with consideration given to ground-water circulation and drainage, and any potential for later carcass exposure. Sprinkle lime or fuel oil on carcasses to discourage uncovering by scavengers and cover the carcasses with at least 3 to 4 feet of soil.

Composting is commonly used for the disposal of some domestic animal carcasses, and it is a technique that can be adapted to wildlife situations. The requirements for composting carcasses include an impermeable surface on which to place composting piles, a roof or other means of controlling moisture in the piles, and raw materials to mix with carcasses to achieve the correct carbon to nitrogen ratio for optimal decomposition of carcasses (Fig. 4.14).

When the combination of animal species, cause of mortality, and local situation allow, carcasses may also be disposed of by an animal rendering plant, and in rare instances infected wildlife may be killed and processed for food. Both of these methods are sometimes used for domestic species and captive-reared wildlife, but conservation laws generally prohibit the processing of free-living wildlife (with the exception of fish) as a commercial food source within the United States. Judgments on the use of rendering and food processing as animal disposal methods should be made only by qualified disease control specialists.

To the extent possible, dispose of carcasses on-site to reduce the risk associated with transporting contaminated material. Regardless of whether burning, burial, or large-scale composting is used, earth-moving equipment is needed. The disease contingency plan should identify how and where bulldozers, backhoes, and similar equipment can be obtained.

Animal Relocation

It is often as necessary to deal with the live, apparently healthy population during disease control activities as it is to remove and dispose of animals dying from disease. Depending on individual circumstances, consider denying animal use of specific sites by dispersing animals from the problem area, concentrating and holding wildlife within a specific area, or trapping animals for sampling.

Scare devices such as propane exploders (Fig. 4.15A) and cracker shells (Fig. 4.15B) may be useful for keeping wildlife away from a toxin or infectious agent within a specific area. Hazing wildlife with airplanes, helicopters, airboats, snowmobiles, and other motorized equipment has also been successful for moving them away from disease problem areas. Conversely, wildlife can be concentrated in an area for euthanasia, and they can be lured to other areas by broadcasting and dumping large amounts of grain and other feed to prevent their movement to problem areas, by knocking down standing grain to make it more available to them, by providing water through pumping operations and diverting water flow, and by providing refuge by closing the area to hunting and other interactions between wildlife and humans (Fig. 4.16). Take care to assure that grain used for attracting wildlife is not moldy and does not contain dangerous concentrations of mycotoxins.

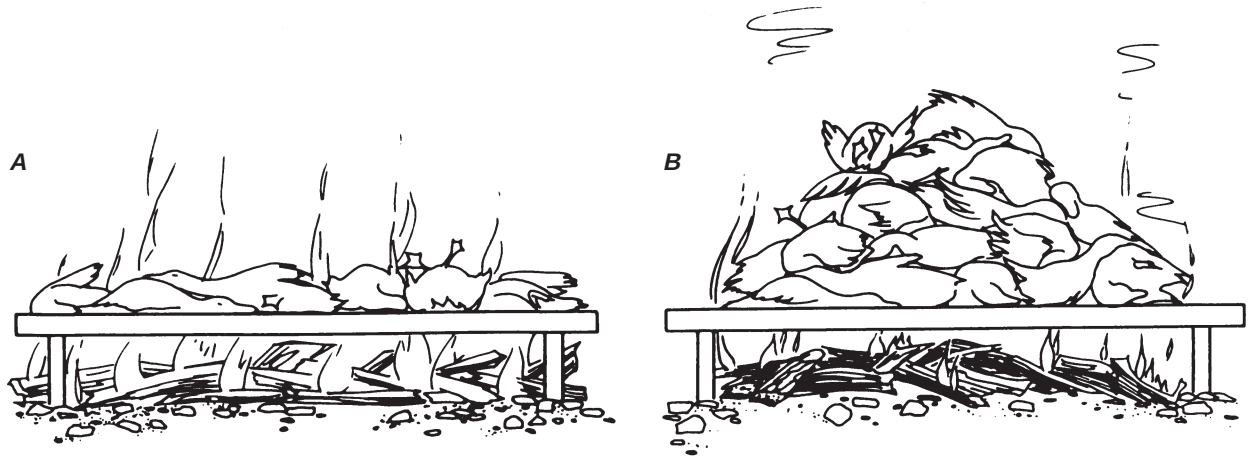


Figure 4.13 *Examples of* ***(A)*** *correct and* ***(B)*** *incorrect layering of carcasses for burning. Carcasses must be burned one layer at a time to prevent charred outer carcasses from insulating inner carcasses from incineration. (Illustration by Randy Stothard Kampen)*

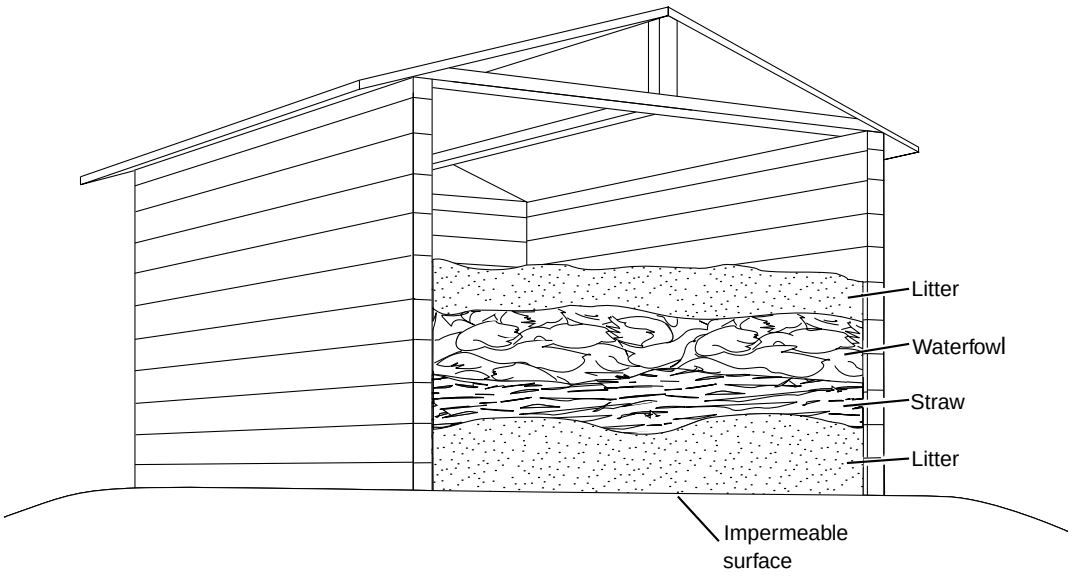


Figure 4.14 *Example of a simple composting bin for waterfowl carcasses. Litter (bedded manure from poultry houses is a good source), straw, and carcasses are added proportionally to achieve the appropriate moisture content and carbon to nitrogen ratio. (Modified from Rynk, 1992.)*

Food and water are also helpful in trapping wildlife for assessing disease control activities. When birds have been lured to a site, they may be captured by such means as drugs incorporated within feed, rocket nets, drop nets, walk-in and swim-in traps, or other means of preventing escape (Fig. 4.17).

A timely response to disease outbreaks can be facilitated if such factors as need for special permits, area closures, possible involvement of endangered species, and water purchase can be anticipated and addressed before an urgent situation arises.

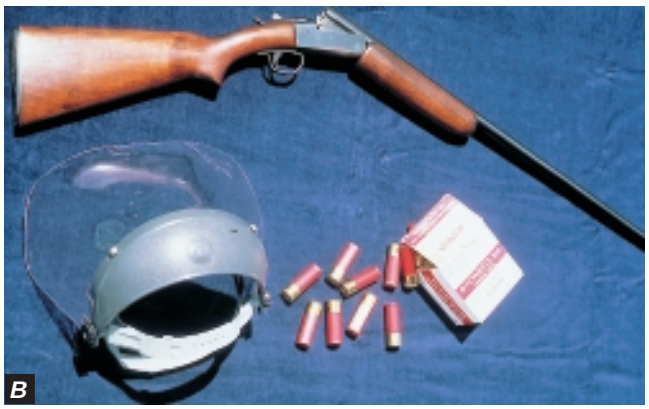
Because of the potential complexity of biological interactions in animal relocation, field managers should seek the advice of disease control specialists whenever possible before taking independent action. As a general rule, animal dispersal is not recommended when infectious disease is involved unless it can be assured that the population being dispersed will not infect other wildlife. Also, it is important that water manipulation not produce conditions favorable to development of botulism or other disease problems.

Disinfection

The purpose of disinfection is to prevent the mechanical transmission of disease agents from one location to another by people, equipment, and supplies. Some viruses, bacteria, and other infectious agents have considerable environmental persistence. Disinfection of the local environment involved in a disease outbreak may be required to prevent recurrence of the disease when the site is used by other animals. Disinfection of a disease outbreak site should always be done under the direct guidance of disease control specialists.

Wash thoroughly the clothing worn during disease control (coveralls and clothes worn under protective raingear) before it is used again. Personnel should shower and shampoo their hair before leaving the site, if possible, but always before they go to other wildlife areas. Disinfect boots before entering vehicles when in contaminated areas, and disinfect all equipment to the extent possible before it is moved from the area (Fig. 4.18A and B). Give special attention to the underside of vehicles (Fig. 4.18C and D). Put motor vehicles through a car wash before moving them to other areas, and wash and clean boats and all-terrain vehicles before they leave the area. Large volume tanks and pumps that can be operated from mobile units such as trucks (Fig. 4.19) and boats are especially useful for holding and dispensing disinfectant.

Disinfection procedures require a suitable disinfectant, containers for that disinfectant once it has been diluted to appropriate strength, and a way of applying the disinfectant. Commercial disinfectants are available from farm supply stores and veterinarians. Refuge managers and other field managers should consider keeping a supply of disinfectant for general use. Chlorine bleach is a highly suitable disinfectant and it is available at most grocery stores. For general



Photos by James Runningen

Figure 4.15 *Wildlife can be discouraged from use of areas by* ***(A)*** *propane exploders that function by the ignition of propane gas within the "cannon" due to the striking of a flint at a timed interval. With the exception of placing the cannon and maintaining a fuel supply, this activity does not require the presence of personnel.* ***(B)*** *Manual firing of cracker shells has also been used successfully to discourage wildlife use of areas. These fireworks-like shells should only be fired through a break-open type shotgun so that the barrel can be checked between shots to assure that there are no obstructions remaining in the barrels. These shells should not be used where they can fall into dry vegetation because of fire hazard.*

Photos by Milton Friend

Figure 4.16 *Closure of areas is often needed to assist disease control operations.* ***(A)*** *Sign used to close Lake Andes National Wildlife Refuge during the duck plague die-off.* ***(B)*** *Sign used to delineate refuge area so that bird disturbance and movement was minimized during another South Dakota disease control operation.*

use, dilute one part chlorine bleach with 10 parts water. Use stronger concentrations of one part bleach to five parts water for disinfecting heavily contaminated areas.

Stiff bristle brushes, buckets, and containers that can be used for foot baths and pressure or hand sprayers that can be used to dispense the disinfectant are also needed. The station contingency disease plan should identify readily available sources of these supplies and equipment.

When the disease problem involves an infectious agent, personnel handling contaminated materials should refrain from working with similar species or those susceptible to the disease for at least 7 days following completion of their disease control activities. For example, a field manager involved in an intensive avian cholera disease control operation on Monday should not band waterfowl in that refuge or elsewhere until Tuesday of the next week.

Personnel

Labor-intensive operations such as carcass removal and disposal sometimes require more personnel than are usually employed on an area. In some instances, specialized help such as low level aircraft flights for surveillance may be needed. The use of nonstation personnel for routine operations has a potential educational value. For example, the use of local sportsmen clubs to help with carcass collections during a major lead poisoning die-off has been highly effective in changing negative attitudes towards nontoxic shot use.

Sportsmen clubs; retriever clubs; biology and wildlife classes at local universities and colleges; local chapters of conservation organizations such as the Audubon Society; the active military and National Guard, who also may provide valuable technical assistance; and similar groups have all provided volunteer assistance in combating disease problems

Photos by Milton Friend

Figure 4.17 *Various types of capture devices are useful for disease operations.* ***(A)*** *Rocket net being fired over Canada geese.* ***(B)*** *Snow geese captured by cannon nets.* ***(C)*** *Constructing a funnel trap to capture birds in a zoological park.**— Figure 4.17 is continued on p. 36.*

Photos by Milton Friend

Figure 4.17—continued (D) *Capturing birds within a funnel trap.* ***(E)*** *Capturing flightless Canada geese in a drive trap.* ***(F)*** *Capturing waterfowl in a large, baited funnel trap.* ***(G)*** *Using drugged grain to capture birds in residential situations. When drugs are used, maintain close surveillance of the situation so that animals that become drugged, such as the bird* ***(H)*** *lying on its back, can be promptly collected before they are seized by other animals or drown if they venture into the water before the drug takes effect.*

G

H

Photos by Milton Friend

Photo by Milton Friend

Photo by Milton Friend

Photo by J. Christian Franson

Photo by Milton Friend

Figure 4.18 *Equipment and personnel should be disinfected to the extent possible before leaving disease operation areas.* ***(A)*** *Initial disinfection procedures should take place well within the contaminated area.* ***(B)*** *Boots and other items in contact with the ground should receive a second application of disinfectant at the point where entry is made into the "clean area," as is being done at the location where the specimen chest is being transferred.* ***(C and D)*** *Various types of spray units can be used to apply disinfectant to the underside of vehicles. Tires and wheel wells are the primary areas of concern as they may contain contaminated soil or animal fecal material from the disease area.*

Photo by Terry Amundson, Wisconsin Department of Natural Resources

Photo by Milton Friend

Figure 4.19 ***(A)*** *Portable tank and pump mounted on a truck bed for dispersing disinfectant during duck plague control operation and* ***(B)*** *application of that disinfectant to a structure used to house birds. The long length of hose on this unit allowed all areas of major bird use to be reached from service and perimeter roads.*

at various times and places. Sound judgment must be exercised in the selection and utilization of volunteers because of legal liability in case of an accident. Contingency plans should list groups and organizations and contact persons for each group, their telephone numbers, and an approximation of the work force and times of its availability (e.g., weekends only or Wednesday only). For technical assistance, list the specific type of personnel needed, such as bulldozer operator or helicopter pilot.

In addition to preparing a station contingency plan, wildlife personnel should become familiar with the other phases of disease control operations. Table 4.1 provides a descriptive outline of these phases. Especially relevant to field managers are the equipment and supply needs identified under the Disease Response Section of Table 4.3.

Response Modifications

Disease control operations can be seriously undermined without current assessment of wildlife morbidity and mortality and the cause of disease problems. When infectious or highly toxic agents are involved, early detection of disease problems is critical to preventing the problem from becoming widespread. Also, failure to accurately assess the cause of the die-off can result in control actions actually contributing to the magnitude of losses and spread of the problem. Different types of disease problems require different types of response. Do not assume that the current die-off is due to the same cause as previous die-offs that have occurred on the area or that only one disease agent is responsible. It is not uncommon for two or more causes of wildlife mortality to occur simultaneously in an area. Control of these different diseases may require opposite types of actions, thereby requiring that a more comprehensive strategy be developed for the disease control operation.

Refuge managers and other field biologists greatly influence the effectiveness of disease control operations by their responsiveness, knowledge of the local situation, how well they are prepared, the flexibility they maintain, their resourcefulness, and when possible, their ability to obtain appropriate technical assistance and training for combating disease problems. Timely and properly carried-out disease control activities can significantly reduce the magnitude of wildlife losses that might otherwise occur. When carrying out control activities, always consider the safety of the personnel involved.

Milton Friend and J. Christian Franson

Supplementary Reading

Friend, M., 1995, Disease considerations for waterfowl managers *in* Whitman, W. R., and others, eds., Waterfowl Habitat Restoration Enhancement and Management in the Atlantic Flyway (3): Dover, Del., Delaware Department of Natural Resources and Environmental Control, p. J24–J117.

Roffe, T.J., Friend, M., Locke, L.N., Evaluation of causes of wildlife mortality, *in* Bookout, T. A., editor, 1994, Research and management techniques for wildlife and habitats, Fifth ed.,: Bethesda, Md., The Wildlife Society, p. 324–348

Rynk, R., ed., 1992, On-farm composting handbook: Ithaca, N.Y., Northeast Regional Agricultural Engineering Service, 186 p.

Wobeser, G. A., 1994, Investigation and management of disease in wild animals: New York, N.Y., Plenum Press, 265 p.

Table 4.1 *Outline of disease control operations.*

I. Planning

A. Identify needs

1. Sources of additional personnel to help during disease emergencies. Potentially, these include
   a. State and Federal agencies
   b. Active military and National Guard
   c. Private conservation agencies
   d. Local sporting clubs
   e. Local universities

2. Sources and availability of equipment and supplies for disease control operations (Appendix C)

3. Special needs
   a. Burning permits
   b. Endangered species consultations
   c. Lodging and meal facilities for work crews
   d. Ability to attract and hold wildlife in site-specific areas by providing food, water, refuge, or other means
   e. Ability to deny wildlife use of specific areas by scaring devices and other means
   f. Ability to capture wildlife for sampling, immunization, or other needs

B. Record biological information

1. Daily and seasonal wildlife movement patterns within the general area
2. Migration patterns and population peaks for major and endangered species
3. Past history of diseases

C. Prepare contingency plan (See Tables 4.2 and 4.3.)

II. Initial Response

A. Identify problems

1. Obtain diagnosis by submitting carcasses to a qualified diagnostic laboratory as soon as mortality or morbidity is evident. (See Chapters 3 and 4 for shipping procedures.)

2. Conduct field investigation to determine extent of problem (i.e., species, number of wildlife, and geographic area involved).

3. Identify special biological, political, or physical considerations associated with problem. Before proceeding further with II. B and C., seek the advice of a specialist.

B. Establish control of area

1. Close affected area, when warranted, to all but authorized personnel.

2. Identify special work areas for disease control activities.
   a. Carcass disposal sites
   b. Laboratory investigations area
   c. Briefing area for news media and staff
   d. Vehicle parking
   e. Assembly areas for arriving workers
   f. Command post

3. Initiate carcass cleanup, but do not dispose of carcasses without guidance from disease control specialists.

Table 4.1 *Outline of disease control operations (continued).*

C. Communications

Notify appropriate agency and nonagency personnel of die-off.

III. Disease Control

A. Response

1. Disease control actions are dictated by the type of disease, environmental factors, species involved, and other circumstances. Typically, actions associated with major die-offs require:
   a. Bringing personnel, equipment, and supplies on-site
   b. Organizing workforce, briefing workers about the problem, and assigning duties
   c. Carcass pickup and disposal
   d. Monitoring cause of mortality to detect changes in the cause of the problem (die-offs often involve more than a single cause and different control actions may be required for these different causes)
   e. Decontamination of personnel and equipment
   f. News media briefing sessions and "show-me" trips[1]

B. Management

1. Disease management activities often involve:
   a. Population manipulation such as removal, controlled movement including relocation and local concentration of wildlife populations, and population dispersal
   b. Habitat manipulation to prevent, attract, or maintain wildlife use of an area

2. Decontamination of the infected environment, such as:
   a. Chemical treatment of land, water, and structures
   b. Vegetation and water removal (desiccation) to allow air and sunlight (ultraviolet) to destroy micro-organisms

C. Controlled burning to remove vegetation and dispose of mechanical structures

IV. Surveillance

A. Monitoring

After disease control operations have ended, the area should be kept under surveillance for 10 to 30 days to watch for additional flareups.

B. Investigations

This stage is also an appropriate time to conduct followup investigations of factors that helped cause and sustain the problem, and to carry out wildlife and environmental sampling to discern disease exposure patterns and environmental reservoirs of disease agents.

V. Analyses

Each disease control operation provides a learning experience. It is important to the success of future operations to evaluate what was done, the degree of success achieved, problems encountered, and what should have been done differently.

[1] Media briefing sessions and "show-me" trips should be conducted by personnel with comprehensive knowledge of the situation.

Table 4.2 *Equipment and supplies used in disease control operations.*

Activity	Equipment and supplies
A. Carcass Collection	
1. Transportation of personnel	a. All-terrain and four-wheel vehicles, snowmobiles b. Airboats, canoes, other boats c. Helicopter d. Waders, snowshoes
2. Transportation of carcasses	a. Large, heavy-duty plastic bags b. Plastic trash cans with lids c. Sleighs and trailers d. Trucks, boats e. Strapping tape and other means of securing closure of containers
B. Carcass Disposal	
1. Burial	a. Earth-moving equipment for digging trenches or pits (bulldozer, backhoe) b. Shovels c. Lime or fuel oil to spread on carcasses d. Any applicable permits
2. Incineration	a. Portable incinerators and fuel b. Local permanent incinerator c. Earth-moving equipment for digging trenches or pits (bulldozer, backhoe) d. Burning permits e. Shovels f. Metal grates and cinder blocks for building burning platforms g. Sheet metal or metal roofing for heat reflectors h. Fuel for burning carcasses (wood, coal, rubber tires, fuel oil, napalm) i. Fire suppression equipment
3. Composting	a. Composting bin made of pressure-treated lumber b. Straw and manure to alternate with layers of dead birds c. Trucks to transport carcasses, straw, and manure
C. Sanitation Procedures	
1. Decontamination of environment	a. Chemical disinfectants and structures b. Pumps and suction apparatus for drainage of water areas c. Buckets, brushes d. Spray application by aircraft, power systems mounted in trucks and boats, and hand-carried spray units
2. Protection of personnel and prevention of mechanical movement of disease agents to secondary locations by people and equipment	a. Raingear, coveralls, rubber gloves, rubber foot gear, hats b. Spray units and chemical disinfectants c. Plastic bags for transportation of field clothes to laundry d. Brushes, buckets e. Disposable gloves, hats, coveralls, and foot coverings

Table 4.2 *Equipment and supplies used in disease control operations (continued).*

Activity	Equipment and supplies
D. Field Communications	
1. Field activities	a. Portable radios or cellular telephones for communication between field personnel b. Radios in vehicles for communication between field units and between units and command post
2. Information activities	a. Word processor or typewriter for preparing briefing documents b. Maps, acetate, and other supplies for overlays depicting die-off and control activity information c. Telephone lines for communication with others d. Transportation for news media “show-me” trips
E. Surveillance and Observation	
1. Field activities	a. Aircraft and pilots certified for low-level flights (500 feet and below) for monitoring wildlife populations and environmental conditions b. Binoculars and spotting scopes
2. Office activities	a. Maps, acetate, and other supplies for tracking the progress of events and wildlife populations associated with die-off b. Telephone for contacting others to trace movement of migrant bird populations that might enter problem area or that have departed problem area
F. Wildlife Population and Habitat Manipulation	
1. Denying wildlife use of an area	a. Aircraft, boats, snowmobiles, and other motorized means of hazing wildlife populations b. Propane exploders c. Cracker shells, break-open shotguns, and protective face shield d. Audio systems and other scare devices e. Pumps for draining water or adding water to areas
2. Concentration and maintenance of wildlife in a specific area	a. Grain and other sources of food b. Pumps and water to provide habitat c. “No Hunting” and “Area Closed” signs to provide temporary refuge area
G. Wildlife Sampling and Monitoring	
1. Wildlife capture	a. Cannon nets and other capture equipment b. Grain and other baits to lure wildlife to capture site
2. Wildlife marking	a. Visible marking devices such as paint, neck collars, and other devices b. Permanent marking devices such as leg bands and ear tags (see Bookhout, 1994) c. Temporary marking devices such as radio transmitters

Table 4.3 *Station disease contingency plan.*

I. Introduction

A. Size, configuration, and other important characteristics of station area conveyed with help of tables, maps, photographs, station brochures, public use maps, and similar documents

B. Record of previous disease outbreaks, including nature of disease, species involved, magnitude of die-off, and season and year (Table 4.4)

II. Disease Surveillance

A. Brief outline of current surveillance activities on station and adjacent areas — State, Federal, and private

B. Identify disease reporting and notification procedures (names, titles, organization, and telephone numbers of persons to be contacted)

III. Disease Response

A. Logistical considerations

1. Personnel sources (telephone numbers, addresses, names of contact persons)
   a. Local, State, and Federal agencies (military, university)
   b. Sporting clubs and volunteers

2. Equipment (types and numbers on-site, and sources off-site)
   a. Vehicles (conventional and all-terrain)
   b. Aircraft (fixed-wing and rotary)
   c. Earth-moving equipment (backhoe, bulldozer)
   d. Pumps (for flooding or draining marshes)
   e. Boats (motor, self-propelled, air boats)
   f. Radios (portable and fixed); during nonfire seasons the
      National Interagency Fire Center
      3905 Vista Avenue
      Boise, Idaho 83704
      (208) 389-2458
      is a potential source for obtaining assistance for very large communication needs
   g. Incinerators
   h. Composting bins
   i. Decontamination units (sprayers)
   j. Scaring devices (propane exploders, sirens)
   k. Freezers
   l. Portable toilets (construction-site type)

3. Supply sources (Identify sources, addresses, and telephone numbers of local or closest sources.)
   a. Disinfectants and chemicals
   b. Plastic bags
   c. Fuel for carcass burning
   d. Field clothes (gloves, rainwear, coveralls, boots)
   e. Plastic trash barrels, tubs, scrub brushes
   f. Scaring devices (cracker shells, fireworks); provide contact telephone number and address for local animal damage control office
   g. Dry ice and liquid nitrogen
   h. Grain and other wildlife foods
   i. Nearest shipping address for air and ground receipt of goods and supplies

Table 4.3 *Station disease contingency plan (continued).*

4. Lodging for temporary personnel assigned to disease control operation

5. Food
   a. On-site capabilities
   b. Off-site capabilities (Give consideration to early and late hours.)

6. Identify working areas (Diagrams are sufficient; limited narrative may also be required; Fig. 4.2.)
   a. Clean areas
      1. Command post (must have adequate telephones)
      2. News media briefing room
      3. Parking
      4. Eating areas
      5. Staff assembly and rest areas
      6. Equipment and supply receipt
      7. Other
   b. Transition areas
      1. Decontamination of personnel
      2. Decontamination of equipment
   c. Contaminated areas
      1. Carcass disposal
      2. Laboratory investigations
      3. Animal holding

B. Biological considerations (Provide data in charts, figures, photographs, maps, tables.)

1. Species and population data
   a. Major species (Identify by season of presence, relative abundance, and peak population periods.)

2. Wildlife movement patterns (Figs. 4.3 through 4.5)
   a. Daily
   b. Seasonal
   c. Production and dispersal patterns

3. Weather patterns
   a. Freeze-up and ice-out periods
   b. Major periods of precipitation and drought
   c. Other (temperature profiles, major periods of haze, fog, and high winds)

4. Habitat and population manipulation potential
   a. Methods (water manipulation capability, feeding)
   b. Anticipated population response to habitat (movement, concentration, dispersal)

C. Communications (Provide lists of principal local and regional contact personnel and telephone numbers.)

1. State agencies
   a. Conservation
   b. Agriculture
   c. Health department
   d. University diagnostic laboratories

Table 4.3 *Station disease contingency plan (continued).*

2. Federal agencies
   a. Environmental Protection Agency
   b. U.S. Department of Agriculture
   c. U.S. Public Health Service

3. Other organizations
   a. Cooperating organizations (e.g., area representatives of Audubon Society, National Wildlife Federation, Ducks Unlimited)
   b. Local sporting clubs
   c. Private wildlife area managers
   d. Local game breeder organizations
   e. Local domestic animal husbandry and production operations

4. Media
   a. Television
   b. Radio
   c. Newspapers

IV. Supplemental Information

A. Location of nearby laboratories (hospitals, universities, county and State facilities)

B. Federal and State permit status for biological collections

C. Burning permits

D. Regulatory requirements

E. Background information (e.g., water sources, water-quality data, potential sources of disease transmission between wildlife and domestic animal concentrations)

F. Identification and location of adjacent or nearby wildlife refuges, management areas, and private reserves

G. Identification of unusual or politically sensitive aspects of area

Table 4.4 *Example of a disease outbreak summary for a wildlife management area[1]. [—, no data available]*

Disease	Date	Location	Principal species involved	Estimated population at risk[2]	Carcass count	Estimated total	Control efforts	Diagnostic laboratory
Unknown	Aug.–Oct. 1972	Unit 6 and Yellowleg Flat on adjacent State land	Northern pintail, teal	5,000	1,500	3,000	None	None
Lead poisoning	April 1980	Mud Lake	Redhead, tundra swan	—	75	200	None	State diagnostic laboratory
Lead poisoning	Dec. 1983	Mud Lake	Mallard, Canada goose	10,000	—	150	Blinds were relocated in 1984	Veterinary Science Dept., State college
Unknown	Jan. 1983	Mud Lake	Muskrat	3,000	100	500	None	Veterinary Science Dept., State college
Avian botulism	July–Sept. 1985	Units 3, 4, 5	Shorebirds, northern pintail, teal	25,000	2,200	10,000	Drained Unit 4, flooded 3 and 5	National Wildlife Health Center

[1] The material in this table is fictitious and for illustration only.
[2] Number of animals using the area involved in the die-off.

Chapter 5

Euthanasia

Background

Euthanasia means to cause humane death. Some current euthanasia techniques may become unacceptable over time and be replaced by new techniques as more data are gathered and evaluated. The following information and recommendations are based largely on the 1993 report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia. The recommendations in the panel report were intended to serve as guidelines, and they require the use of professional judgement for specific situations. Ultimately, it is the responsibility of those persons carrying out euthanasia to assure that it is done in the most humane manner possible.

There is no perfect euthanasia technique appropriate to all situations. What is sought in each instance is immediate insensitivity of the animal to pain as a result of depression of the central nervous system (brain and spinal cord). The AVMA panel in its evaluation considered the following to be important factors to consider when selecting a euthanasia method:

Considerations for selecting a euthanasia method

- Does the method cause the animal to lose consciousness and die without causing the animal pain, distress, anxiety, or apprehension?
- How much time does the method require to induce unconsciousness?
- Is the method reliable?
- Does the method put personnel at risk of injury or health problems?
- Is the method irreversible?
- Is the method compatible with the purpose of euthanasia?
- Will the method cause distress and anxiety among observers and personnel?
- Does the method interfere with or detract from the subsequent evaluation, examination, and use of tissue?
- Are drugs required by the method available? Can the drugs be abused by humans?
- Is the method appropriate for the animal age and species?
- Is the equipment required by the method in proper working order?
- Is the method cost-effective?

Methods of euthanasia are physical or chemical. Physical methods of euthanasia include cervical dislocation, decapitation, stunning and removal of blood, and gunshot. Chemical methods of euthanasia involve introducing a toxic agent into the body by injection or inhalation. After completing euthanasia, be certain that specimens being collected are properly identified, preserved, and packaged for transportation to the diagnostic laboratory (see Chapter 2, Specimen Collection and Preservation, and Chapter 3, Specimen Shipment). Be sure to indicate the euthanasia technique used.

Physical Euthanasia

Cervical Dislocation

Cervical dislocation can be used without any special equipment to euthanize small birds and ducks. The dislocation must take place at the base of the brain, or within the upper one-third of the neck (the cervical spine). Grasp the base of the bird's skull in one hand and its body, usually at the base of the neck, in your other hand. Pulling rapidly and firmly in opposite directions will separate the spinal cord (Fig. 5.1). Cervical dislocation can be used for larger birds, like geese, by separating the upper cervical spine with an emasculatome, which is available from veterinary supply

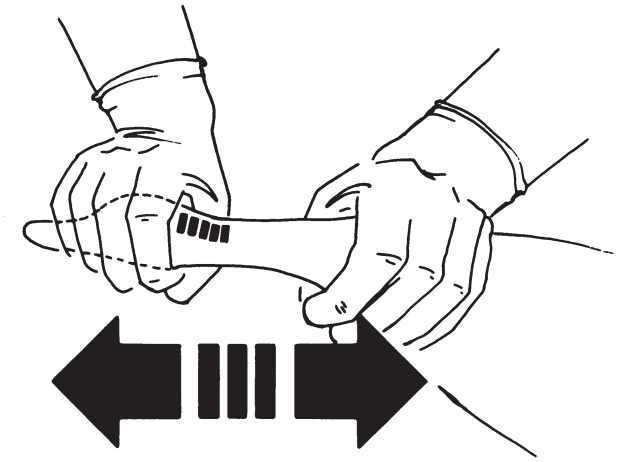


Figure 5.1 *Cervical dislocation procedures. The brain can be separated from the spine in small- to medium-sized animals by grasping the animal at the base of the skull with one hand, at the base of the neck with the other, and pulling rapidly and firmly in opposite directions with a strong snapping action.*

stores. As with all methods, learn how to properly use this instrument before applying it to a live animal.

Cervical dislocation may upset the casual observer because animals, especially birds, convulse for several seconds to minutes after death. These movements are due to spinal reflexes and the animals do not feel pain. This technique is effective, rapid, inexpensive, and only minimally affects diagnostic testing.

Decapitation

Severing the head from the neck is an effective method of euthanasia for small mammals and any size bird, but it is often used for larger waterfowl. Use a knife, machete, hatchet, or bolt cutters to ensure that the spinal cord, encased in the cervical spine, is severed. The same convulsions seen after cervical dislocation will follow decapitation. This technique has similar attributes as cervical dislocation. However, take care to prevent injuries to personnel resulting from the use of the sharp implements, and to prevent exposing personnel to toxic or infectious agents that may be in the blood.

Stunning and Exsanguination (Removal of Blood)

This method requires striking the center of the skull to render the animal unconscious, followed by severing the major blood vessels in the neck, and allowing the animal to bleed out. Do not use this technique if the brain is required for diagnostic tests.

Gunshot

Shooting animals in the head, or the neck if the brain is needed for diagnostic purposes, with a small caliber rifle can be used as a method of euthanasia. Training and experience are required to assure a humane death, and also to reduce the human safety hazards.

Chemical Euthanasia

Extreme caution is required for the use of chemical euthanasia, because of the potential hazards for humans. These procedures should be carried out only by trained individuals who are properly authorized to use the appropriate chemicals.

Inhalant Anesthetics

Several inhalant anesthetics have been used for wildlife euthanasia. Halothane is often the inhalant selected because it rapidly induces unconsciousness. Enflurane also rapidly induces unconsciousness, but seizures under deep anesthesia from enflurane are more common than from halothane. Methoxyflurane vaporizes slowly and, therefore, has a longer anesthetic induction time, which can cause the bird to become agitated. Isoflurane has a rapid induction time, but its odor can cause the animal to hold its breath, thereby delaying unconsciousness. Nitrous oxide has a low potency and is available only in gas form; other anesthetics are purchased as a liquid, and they vaporize at room temperature and normal air pressure. Nitrous oxide can be used in combination with other inhalants to speed anesthesia, but it should not be used alone because animals often become agitated and distressed before they lose unconsciousness.

To administer an inhalant anesthetic for euthanasia of an individual bird, prepare a cone (from a syringe case or other plastic material) that will fit snugly when it is placed over the beak and nares (Fig. 5.2). Pour a small amount of the anesthetic agent on a piece of cotton, tissue, or cloth, and place it in the narrow part of the cone. Restrain the bird; put the open end of the cone over the beak and nares, and continue restraining the bird until it becomes unconscious. Restraint can then be discontinued, but keep the cone in place for several minutes before checking to assure that the bird is dead. Alternatively, place an individual bird, or several small birds, in a cage or crate; cover it with plastic or place the cage in a covered plastic barrel. Place the cotton, tissue, or cloth soaked with anesthetic agent inside the chamber with the birds and tie or otherwise seal the plastic to prevent the vaporized agent from escaping (Fig. 5.3). Cold temperatures will decrease the rate at which the liquid becomes gas. Small mammals can be euthanized by similar procedures.

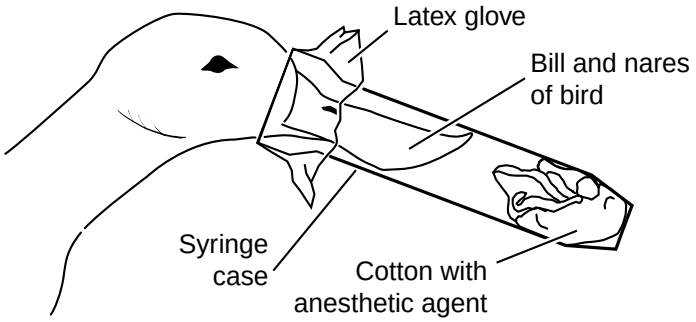


Figure 5.2 *A cone prepared from an empty syringe case can be used for euthanasia. Tape a piece of latex glove over the open end of the cone, and cut a slit in the latex so that the bill and nares fit through it. Place the anesthetic agent on a piece of cotton in the end of the cone.*

A animal exposed to anesthetic gas may pass through an "excitation phase" before it becomes unconscious; it may vocalize and appear to struggle for a short time. This behavior may be distressing to the casual observer and it can be dangerous for the handler, depending on the species. It is important to assure that the animal is dead, and not just unconscious, before shipment, necropsy, or disposition. After removing the animal from the gas environment, it may wake up quickly, with little warning. Remember this when working with raptors, carnivores, and other biting animals.

Because all of these gases constitute a human health hazard, including the potential to cause spontaneous abortion and congenital abnormalities, the workplace must be well-ventilated.

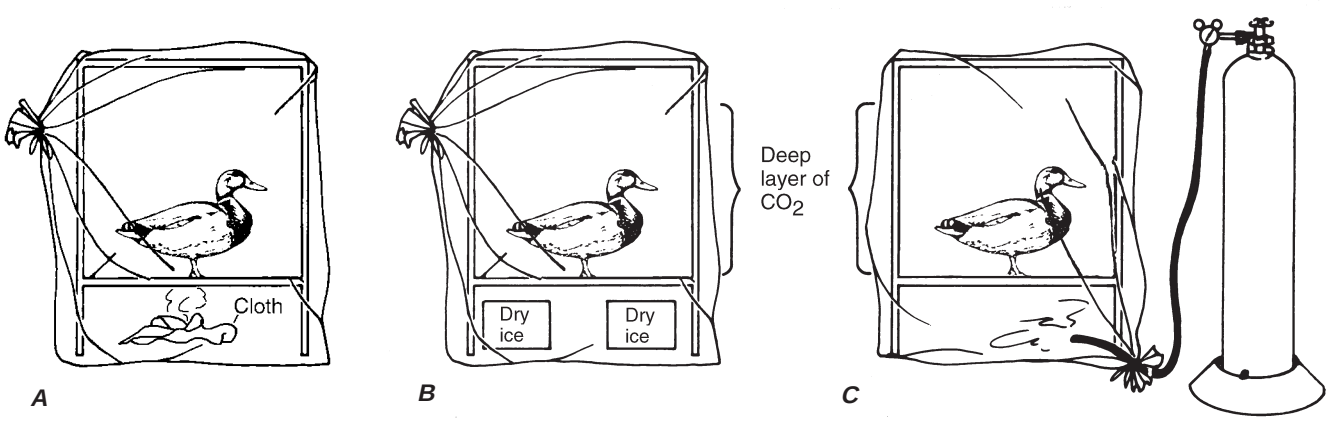


Figure 5.3 *Use of cage enclosed with plastic for euthanasia of birds.* ***(A)*** *Anesthetic agent placed on a piece of cloth under the cage.* ***(B)*** *Evaporation of dry ice.* ***(C)*** *Direct application of carbon dioxide gas. Because this gas is heavier than air, a deep layer of gas must be built up so that the animals being euthanized cannot get above the gas. The chamber containing the animals must not be airtight or gas buildup may result in an explosion. Openings should be at the top of the chamber.*

Toxic Gas

Toxic gases such as carbon monoxide (CO) or carbon dioxide (CO_2) may be useful when many small birds or animals must be killed. Keep in mind that, even at concentrations of less than l percent, carbon monoxide is lethal and represents a substantial human safety hazard because it is highly toxic and difficult to detect. In concentrations exceeding 10 percent, carbon dioxide can be flammable and explosive. Work with this gas, as with anesthetic gases, must be conducted in an open area away from electrical equipment.

Carbon monoxide and carbon dioxide may be purchased as compressed gases in cylinders. Dry ice can also be used as a source of carbon dioxide. If dry ice is used, protect animals from contact with it. Cages covered with plastic bags (Fig. 5.3) or plastic garbage cans can be used as killing chambers, but the cages must be vented to allow displacement of air within the chamber by the toxic gas. Leave the animals in the chamber until breathing and heartbeat have ceased.

Lethal Injection

To administer lethal injections, personnel must be trained in injection techniques and proper doses as well as in the safe handling and disposal of needles, syringes, and drugs. Federal drug regulations make the use of these agents, except by licensed veterinarians, largely impractical. Lethal injections can be used for any animal that can be given an intravenous injection, but they are probably most useful for mammals and large birds, such as geese.

Sleepaway® (made by Ft. Dodge Laboratories, Inc., Ft. Dodge, Iowa) and Beuthanasia – D Special® (made by Burns-Biotic Laboratories, Inc. Omaha, Neb.) are concentrated barbiturate solutions plus additives. The solutions are inexpensive, but, due to the potential for human abuse, require licensing by the Federal Drug Enforcement Administration (DEA) for purchase, use, and storage. Considerable record-keeping of use of the drug is required by the DEA.

Lethal injections may not be appropriate in certain instances because drug residues interfere with some tests. Check first with the diagnostic laboratory to see if the proposed euthanasia technique is compatible with the testing to be performed.

The need for individual handling and injection of each animal generally precludes using this technique for euthanasia of more than a few birds or animals per event. Proper disposal of carcasses is needed to prevent secondary poisoning of scavenger species in situations where more birds or animals are euthanized than are needed for diagnostic testing.

J. Christian Franson
(Modified from an earlier chapter by Patricia A. Gullet)

Supplementary Reading

Andrews, E.J., Chairman, 1993 Report of the American Veterinary Medical Association panel on euthanasia, 15 January 1993: Journal of American Veterinary Medical Association 202:229–249.

Chapter 6

Guidelines for Proper Care and Use of Wildlife in Field Research

Prologue

Public attitudes towards animals continue to change over time. These changes apply to wildlife along with other species, and in recent years, attitudes have been increasingly oriented toward assuring that all species receive proper care whenever human interactions are involved. Guidance regarding the application of euthanasia is provided in the previous chapter. This chapter provides basic guidelines for the proper use of wildlife in field investigations. We believe this previously published information from The Wildlife Society is sufficiently important to include in this field manual. The Wildlife Society has been kind enough to grant permission for this reproduction. The scope of this chapter extends to all wildlife, and the application of this material extends beyond research to all wildlife investigations. This chapter is reproduced, with the addition of illustrations and minor modifications, as it appeared in Research and Management Techniques for Wildlife and Habitats (Bookhout, 1994), and, thus, it deviates from the format for the rest of Volume I.

Introduction

Philosophy

Scientists do not operate in a vacuum, but rather in an arena with responsibilities to the organisms they study and to society. Professional scientists must consider the effects of their activities on the organisms under study, on the validity of study results, and on the use of these organisms by other segments of society. The Wildlife Society recognizes these relationships and supports the sound application of responsible methods for the conduct of animal research in all field and laboratory investigations. This position reflects our ethical and moral concerns regarding human interactions with each other and with other species, and recognizes the scientific benefits of investigations that are not compromised by the manner in which animals are handled or maintained. These concerns are the foundation for our philosophy that responsible methods of animal investigations must include all animal species. Wildlife professionals are urged to apply high standards of animal care and maintenance, and responsible methods of experimental procedures, in conducting each animal investigation.

Purpose

These guidelines are intended for field research involving wild animals. The variety of wild vertebrates investigated and of conditions encountered precludes provision of specific information applicable to each situation. Lists of useful references for those seeking more specific information are provided in the Appendices.

Background

The Animal Welfare Act (7 U.S.C. 2131, and following) was enacted on 23 December 1985, with amendments including Parts l, 2, and 3 (9CFR); Fed. Register 4(168) 3611236163, effective 30 October 1989. The Act established definitions of terms (Part l) used in the regulations (Part 2) and standards (Part 3) for the humane handling, care, treatment, and transportation of regulated animals used for research or exhibition purposes, sold as pets, or transported in commerce. Excluded from the provisions of the Act are cold-blooded vertebrates, birds, rats *(Rattus)* and mice *(Mus)* bred for use in research, horses and other farm animals used or intended for use as food and fiber, and livestock and poultry used or intended for use in improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber. Also excluded are field studies as defined by the Act, i.e., "any study conducted on free-living wild animals in their natural habitat, which does not involve an invasive procedure, and which does not harm or materially alter the behavior of the animals under study." Collection of blood samples, ear-notching, branding, and collection of routine weight and measurement data are examples of exempted activities.

Exclusion of animal species under the Act removes reporting requirements and reduces oversight by the U.S. Department of Agriculture, but does not negate coverage of these species under guidelines established by other agencies. Thus, fish, amphibians, reptiles, birds, and mammals are covered by the National Science Foundation (NSF) and the National Institutes of Health (NIH) guidelines. This coverage is extended to research grants funded by these agencies and to Federal agencies, such as the U.S. Fish and Wildlife Service, that function under the guidelines of the Interagency Research Animal Care Committee.

Role of Institutional Animal Care and Use Committees

A major requirement of the Animal Welfare Act and NIH/NSF guidelines is establishment of institutional facility Animal Care and Use Committees (ACUCs). The function of ACUCs is critical to the conduct of scientific investigations. Each ACUC must consist of at least three members, one of whom is the attending veterinarian of the research facility (or another veterinarian with delegated program responsibility) and one of whom is not affiliated in any way with the facility other than as a committee member. The purpose of the ACUC is to evaluate the care, treatment, housing, and use of animals and to certify compliance with the Act. This process involves evaluation of experimental protocols to ensure that animal pain and distress are minimized. ACUC oversight includes laboratory and field studies. Consensus recommendations on effective ACUCs for laboratory animals were provided by Orlans and others (1987). Differences between laboratory and field studies (Orlans, 1988) do not negate the need for application of responsible methods for care and use of animals during field research activities. ACUCs and field investigators must work together in reaching agreement on appropriate protocols and methods for specific circumstances of the field research to be undertaken. "Standards for humane treatment of wild vertebrates must continue to be constantly developed, applied, and re-examined. Practices that are acceptable today may well prove unacceptable to tomorrow's scientific community, and/or to society in general" (Canadian Council on Animal Care, 1984, p. 192). Wildlife professionals are strongly encouraged to serve on ACUCs and contribute their specific knowledge about the needs of free-living wildlife to help guide Committee actions involving protocol reviews for field investigations. Wildlife professionals also are encouraged to publish manuscripts that document the proper care and maintenance of free-living wildlife species during field investigations. Development of this information by knowledgeable field biologists provides specific species information for guiding ACUC decisions involving protocol reviews.

Field research study conditions for wildlife

Irrespective of the species or circumstances involved, wildlife professionals should satisfy the following conditions for all field research studies. Written assurance that these conditions will be met is a prerequisite for project consideration and funding by many granting agencies. These conditions also are principal points for evaluation by the ACUC.

1. Procedures employed should avoid or minimize distress to animals consistent with sound research design.

2. Procedures that may cause more than momentary or slight distress to animals should be performed with appropriate sedation, analgesia, or anesthesia, except when justified for scientific reasons in writing by the investigator in advance.

3. Animals that otherwise would experience severe or chronic distress that cannot be relieved will be euthanized at the end of the procedure or, if appropriate, during the procedure.

4. Methods of euthanasia will be consistent with recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (Andrews and others, 1993) unless deviation is justified for scientific reasons in writing by the investigator. However, species differences must be considered. As noted elsewhere, "The AVMA recommendations cannot be taken rigidly for ectotherms; the methods suggested for endotherms are often not applicable to ectotherms with significant anaerobic capacities" [American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL), and the Society for the Study of Amphibians and Reptiles (SSAR), 1987, p. 2].

5. Living conditions of animals held in captivity at field sites should be appropriate for that species and contribute to their health and well-being (Fig. 6.1). Specific considerations include appropriate standards of hygiene, nutrition, group composition and numbers, provisions for refuge and seclusion, and protection from weather and other forms of environmental stress. The housing, feeding, and nonmedical care of these animals must be directed by a scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. Some experiments (e.g., competition studies) will require the housing of mixed species, possibly in the same enclosure. Mixed housing also is appropriate for holding or displaying certain species.

Photo by Joshua Dein

Photo by J. Christian Franson

Figure 6.1 ***(A)*** *Temporary "field hospital" for recovery of waterfowl with avian botulism and* ***(B)*** *a more permanent structure used for the same purpose. The permanent structure provides shade and has a cement floor for easy cleaning and disinfection and has a water trough the birds can swim in. For both situations, periodic inspection of the pens during each day is needed for the detection and prompt removal of dead birds. Prolonged use of the temporary hospital should be avoided because of fecal contamination that cannot be readily neutralized. By segmenting the temporary facility into separate pens, "pasture rotation" followed by treatment of vacated areas can help provide reasonably clean holding areas. An alternative would be to construct pens that can be easily moved. A tarpaulin or other covering placed over the top of the temporary structure or placement of such structures under the shade of trees will enhance bird survival by minimizing heat stress.*

Wildlife Observations and Collections

General

Before initiating field research, investigators must be familiar with the target species and its response to disturbance, sensitivity to capture and restraint, and, if necessary, requirements for captive maintenance to the extent that these factors are known and applicable.

To the extent feasible, animals with dependent young should not be removed from the wild unless the young also are collected or removed alive and provided for in a manner that facilitates their survival beyond the period of dependency. Whenever possible, voucher specimens of animals, their tissues, and parasitic and microbial fauna collected during field investigations should be deposited in catalogued scientific collections available to others within the scientific community, to provide for maximum use of animals collected.

The number of animals required for investigations depends on questions being investigated, but provision of adequate sample size is essential to assure scientific validity of results and avoid unnecessary repetition of studies. Removal of animals from a population (either for translocation or by lethal means) should be restricted to the fewest animals necessary to achieve established goals, but should never jeopardize the population's well-being.

Investigator Disturbance and Impacts

Potential gains in knowledge from field investigations must be balanced against the potential adverse consequences associated with the conduct of the study (Animal Behavior Society/Animal Society for Animal Behavior, 1986). A high level of sensitivity to the potential, indirect effects of investigator presence and study procedures must be maintained, and appropriate steps must be taken to minimize these effects. Examples of secondary impacts associated with field investigations may include nest desertion, abandonment of young, increased vulnerability to predation, traumatic injuries and mortality resulting from panic escape response, cessation of breeding activities, increased energy use by disrupted species, altered feeding behavior, habitat abandonment, long-term marring of fragile habitats, increased vulnerability to hunting, introduction of disease, and spread of disease. These effects may impact either research (target) or other (nontarget) species. Investigators should use available information on secondary impacts as a basis for taking appropriate precautions to minimize known potential impacts.

Such factors as frequency and timing of investigator presence can influence greatly research effects on target and nontarget species. When applicable, remote methods of data collection can be used to minimize disturbance. Also, habitat conservation should be practiced rigorously during all field investigations, and every reasonable effort should be made to leave the study area and access to it as undisturbed as possible.

Museum Collections and Other Killed Specimens

Collection of animals often is an essential component of field investigations. These collections may involve systematic zoology, comparative anatomy, disease assessments, food preference studies, environmental contaminant evaluations, and numerous other justifiable causes and scientific needs.

Assessment of the need should involve appropriate evaluations to determine that the proposed collections will provide scientific data that are not duplicative of information already available in the scientific literature (unless confirmation of these data is needed), or that are presently available in accessible scientific collections and repositories. These evaluations also should assess whether suitable information can be obtained from alternative methods that do not require taking live animals. Methods of collection must be responsible, minimize the potential for the taking of nontarget species, and not compromise the purpose of the study. In some instances it is possible and practical to capture animals and then apply approved euthanasia methods (see Andrews and others, 1993). However, for many field studies the only practical means of animal collection are those involving direct killing as the initial step in the collection process. Under these conditions, methods of vertebrate collection must be as species or age-class specific as possible. Methods must not be employed that compromise data evaluation. Appropriate provisions also must be made for proper collection and preservation of biological materials associated with the purpose of the study. Improperly collected or preserved specimens that fail as useful and valid sources of scientific information negate the purpose of collecting the animals.

When shooting is the collection method, the firearm and ammunition should be appropriate for the species and purpose of the study. The shooter should be sufficiently skilled to be able to kill the animal cleanly. If an animal is wounded, immediate attention must be given to appropriate follow-up actions to kill it quickly. Attention also must be given to the animal's location to assure it can be killed cleanly and that it will be readily accessible for retrieval and data collection.

Kill traps, with attendant baits and attractants, are acceptable and effective for animal collection when used in a manner that minimizes the potential for collecting nontarget species. All traps should be checked regularly, at least daily, to prevent specimen loss from scavengers and predators and should be rendered nonfunctional when not in use.

Live traps for nocturnal species should be set before dusk, checked as soon as possible after dawn, and closed during the day to prevent capture of nontarget species. Live traps for diurnal species should be shaded or positioned to avoid full exposure to the sun. Live traps for nonfossorial mammals should enclose a volume of space adequate for movement within the trap; for fossorial mammals, trap diameter should approximate that of the burrow. The live-trap mechanism should not cause serious injury to the animal, and trap

doors should be effective in preventing the captive animal from becoming stuck or partially held in the door opening (Ad Hoc Committee on Acceptable Field Methods in Mammalogy, 1987). Pitfalls used as live traps should contain adequate food to last until the next trap check and should be covered to keep out rain or punctured to permit drainage.

Blood and Tissue Collections

Only properly trained individuals proficient in the required techniques should attempt to take tissue samples from live animals. Collection of tissue samples requires proper animal restraint to avoid traumatic injuries to the animal and to the investigator taking the samples. Use of anesthetics is required when the sample procedure will cause more than slight or momentary pain. The institution/facility ACUC is the proper source for evaluating collection methods and use of anesthetics for noninvasive and invasive procedures for tissue collections from live animals.

Blood is the most common tissue sampled from live animals. A conservative rule of thumb is that the amount of blood drawn at one time from a healthy animal that is to be kept alive should be no more than 1 percent of its body weight. However, the amount of blood taken should be limited to actual needs, rather than the maximum amount that can be safely taken, to reduce stress from handling. Appropriate equipment (e.g., needle size) and sample site should be selected to provide the amount of blood needed for the species involved.

The three most common sites for bleeding birds are the jugular vein of the neck, medial-metatarsal vein of the leg, and brachial vein of the wing (Fig. 6.2). The jugular is preferred for bleeding most birds because of its accessibility and size and the relative ease with which large samples can be taken. The medial-metatarsal vein is not recommended for use in raptors, nor is the brachial vein in large birds such as cranes. Feathers should not be plucked to locate these veins. Birds also can be bled from a variety of other sites including the heart and occipital venous sinus. However, there is seldom reason to assume the risk associated with these sites for nonlethal sampling, even though successful application of these techniques has been demonstrated.

Multiple sites also are available for drawing blood samples from mammals (Fig. 6.3A). Venipuncture of the cephalic,

Photo by Joshua Dein

Photo by Milton Friend

Photo by Joshua Dein

Photo by Milton Friend

Figure 6.2 *Blood can be drawn from a variety of sites and not jeopardize the well-being of birds when properly trained investigators utilize appropriate techniques and equipment for that task.* ***(A)*** *Proper restraint for jugular bleeding of small birds is shown and is best accomplished by the person doing the bleeding.* ***(B)*** *For larger birds such as this blue goose, the handler supports the body weight and restrains the wings by cradling the bird against her body while controlling the head with her other hand.* ***(C)*** *The bleeder normally controls the leg that blood is being drawn from when the medial-metatarsal vein is used.* ***(D)*** *Bleeding from the brachial vein. Care must be exercised so as not to apply excessive torque to the wing.*

femoral, tarsal, or jugular vein, the orbital sinus, or various venous plexuses are common procedures. In some instances cardiac bleeding also is acceptable. Need for anesthesia with any of these procedures depends upon methods of restraint, species being bled, physical condition of the animal, and volume of blood needed. In reptiles, such as turtles, sites for blood collection are more limited (Fig. 6.3B).

Restraint and Handling

General

Safety of both wild animals and scientists who are studying them should be the primary consideration when physical contact between them is judged to be necessary and unavoidable. Nondomesticated animals almost without exception will try to elude capture, handling, and restraint. The means by which a particular animal may try to prevent capture will vary with the species, sex, physiologic condition, and temperment of the individual. In attempts to elude capture, wild animals are capable of inflicting severe damage to themselves and their potential captors.

Behavioral characteristics of wild animals often may be used to assist the potential captor. For instance, animals in a small pen or cage often voluntarily will enter a smaller container to hide and evade capture. If that container provides adequate restraint, the potentially dangerous work of securing the animal can be accomplished more easily. Every effort involving contact between wild animals and humans should be carefully conceived and skillfully executed. Personnel involved must know the habits and behaviors of the animal to be handled; the plan must have suitable alternatives; and a genuine regard for the physical, physiological, and psychological welfare of the animal must be of deep concern to those actually handling the animals. If the planned and alternate procedures do not appear to be satisfactory, the responsible thing to do is cease immediately and return to the planning stage. Trying to enforce unworkable procedures in a particular situation is a virtual guarantee of injury to either the animals or the humans involved.

Physical Restraint

For many situations physical restraint is the most appropriate method of animal handling, because of risks from chemical immobilization to the animal and humans when potentially toxic drugs are used. When physical restraint is selected, an adequate number of sufficiently trained and equipped personnel must be available to complete the task safely. Location and type of capture, as well as procedures to be performed and time required to accomplish them, will influence the particular type of physical restraint. Gloves, catch poles, ropes, nets, body bags, holding boxes, corrals, squeeze chutes, or more sophisticated mechanical holding devices may be required for specific situations (Fig. 6.4).

For some highly excitable or anatomically fragile species, prolonged physical restraint without some chemical tranquilization may result in self-inflicted trauma, physiological disturbances, or, occasionally, death. Investigators have an obligation to make every effort to avoid physical restraint procedures that result in cardiogenic shock, capture myopathy, and other stress-induced causes of mortality in their animal subjects (Fig. 6.5). Stress-related damage may not be immediately apparent but may lead to debility or death after release.

Chemical Restraint

Use of chemicals or drugs to render a wild and potentially dangerous animal safe to handle has many applications in wildlife research and management (Pond and O'Gara, 1994). Use of anesthetics, analgesics, and sedatives is mandatory for the control of pain and distress before potentially painful procedures such as surgery are performed on animals. Use of drugs and "tranquilizer guns," however, is not the panacea to wild-animal restraint. Chemicals used for tranquilization and immobilization, if not correctly handled and delivered, may be dangerous to the target animals and humans (Fig. 6.6). In addition, during the drug induction phase or during recovery, an unrestrained animal may be subject to increased potential for accidental injury or death including predation. While under the effects of the drug the animal may become hyper- or hypothermic, depending on chemicals used and ambient temperature, it may vomit and aspirate the vomitus, or pregnant females may abort. A darted animal may be able to elude its captors and hide before being completely anesthetized, a particularly acute hazard when chemicals are employed that require administration of an antidote. All of these circumstances and possibilities must be understood and evaluated by the researcher before a chemical is selected as the best method of restraint in a given instance.

If chemical restraint is selected, it is imperative for all members of the capture team to have a working knowledge of the chemical or drugs being used, even if they are to be handled and delivered by a veterinarian. It also is the responsibility of researchers to know the effects, side effects, advantages, and disadvantages of the drugs being used, and to have knowledge of such factors as the minimum and maximum induction times and potential for adverse drug reactions. This type of information is necessary to evaluate the danger to target animals, and to humans that might be exposed to the drugs. Searchers should be capable of monitoring the condition of anesthetized animals and be able to apply resuscitative routines in a life-threatening emergency. Specific recommendations for drug use and their dosage, drug delivery systems, and physical restraint techniques applicable to the specific species are available in the published literature (Pond and O'Gara, 1994). Information on use of these methods exists in guidelines on acceptable field techniques by various professional societies (See "Professional society guidelines" at the end of this chapter).

Photo by Joshua Dein

Photo by James Stewart

Figure 6.3 ***(A)*** *Blood collection from the tarsal vein of a deer and* ***(B)*** *from the tail vein of a tortoise.*

Photo by Milton Friend

Photo by Doug Mader

Figure 6.4 ***(A)*** *Squeeze chutes and head restraints can allow a blood sample to be safely taken from the jugular of large ungulates.* ***(B)*** *Poisonous animals such as this rattlesnake should only be handled by well-trained personnel that have experience with these types of species.*

Photos by Julie Langenberg, International Crane Foundation

Figure 6.5 ***(A)*** *The pale coloration of the muscle tissue of the right leg and discolored areas of muscle tissue in the left leg of this whooping crane are lesions of capture myopathy due to stress associated with improper/extended restraint.* ***(B)*** *The light area in this piece of leg muscle from an antelope is also due to capture myopathy.*

Photo by Milton Friend

Figure 6.6 *Extensive tissue damage and hemorrhage, such as seen in the tissues of this black bear, can occur from immobilization with a CO_2 projected dart.*

Animal Marking

Developing means of reliably identifying individual animals to achieve field research objectives often is necessary. In addition to requiring individual identification, researchers may need information on nonconspicuous aspects of physiology or movements, or other aspects of animal ecology that can be determined directly or indirectly through specially designed markers.

Consideration for animal marking

Before initiating any marking procedure for wild animals, researchers must resolve the following questions to determine whether marking is required and appropriate for the particular situation.

1. Do naturally occurring differences in the morphology of the animals under consideration provide sufficient identification to achieve research objectives?

2. How may animals must be individually identifiable?

3. If animals must be physically marked, can a sufficient number of animals be marked in the time available?

4. Are the risks (to both the animal and researcher) associated with capture, handling, and marking, and subsequent well-being, minimal and acceptable in both responsible and scientific contexts?

If the marking process causes pain or distress, as defined by the Animal Welfare Act, appropriate analgesics or anesthetics should be used.

Criteria for Marking

When answers to the four initial questions lead to a decision to initiate an animal-marking program, researchers must search among a wide array of potential techniques with varying strengths and weaknesses to select the method(s) most suited to their particular project (Nietfeld and others, 1994). Technological and methodological constraints and available resources can vary widely from project to project and will require each researcher to examine each potential marking technique in terms of a standard set of criteria. Specific criteria relate to impacts of marking on the organism, validity of the study, and other constraints such as legal requirements.

Evaluation criteria for marking techniques

The following are essential criteria for evaluation of marking techniques:

1. Marks should have minimal effect on the anatomy and physiology of the organism, i.e., no immediate or long-term physical hindrance.

2. Marks should not influence the organism's behavior, i.e., they should not reduce an organism's ability to secure food or inhibit breeding activity (unless the marks are intended as a reproductive inhibitor).

3. Marks that make an organism more conspicuous must be evaluated carefully to ensure that they neither cause others of the same species to react differently to it than to other conspecifics nor subject it to increased selection by potential predators (unless this is a purpose of the study) (Fig. 6.7).

4. Marks should be retained for the minimal period required to achieve project goals.

5. Unambiguous marks that are quick and easy to apply should be selected to avoid extensive handling or error potential.

6. Marks must comply with Federal, State, and other agency rules and regulations.

The first three criteria focus on the well-being of the organism being studied and the potential for marks to influence research results by affecting the fitness or behavior of the organisms. Criteria 4 and 5 may affect the validity of the research design, and criterion 6 reflects other constraints placed upon the researcher. Violation of any of the first five criteria may result in biased research results, so researchers should specifically address these criteria in any evaluation of research resulting from a sample of marked organisms.

Although marks that may be applied to organisms are commonly perceived as passive and visual, markers also

Photos by Milton Friend

Figure 6.7 ***(A)*** *Color marking waterfowl should be done with rapidly drying paints and* ***(B and C)*** *the painted feathers held separated until the paint dries to prevent the feathers from sticking to one another and hindering normal flight.*

exist that are active and visual (lights), that are auditory, that feature radiotelemetry, or that rely on chemical detection. A vast literature exists of techniques and potential concerns regarding the marking of organisms from insects to whales, and it has been summarized in detail elsewhere (see "Professional society guidelines"; Day and others, 1980; Orlans, 1988).

Other Professional and Ethical Considerations

Many organisms of interest to wildlife professionals are free-ranging and may be enjoyed by other segments of society in many ways, from observation or photography to harvest as meat or trophies. Professional ethics dictate that those other potential uses of organisms be considered and accommodated insofar as possible. Wild animals and birds are valued in part because they are wild, and the presence of human-caused marks may detract from that value. Accordingly, short-lived and inconspicuous marks should be selected whenever they can meet the objectives of proposed research. Scientists have an ethical responsibility to attempt to remove collars or other external markers at the conclusion of their research if possible and feasible. Furthermore, professional and ethical considerations dictate that permanent markers that injure or change the appearance of an animal (e.g., toe-clipping, brand-

ing, and tattooing) be employed only under the most humane conditions and when alternate methods are not available to achieve desired research objectives.

Housing and Maintenance of Field Sites

General

Proper care and responsible treatment of incarcerated animals must depend on scientific and professional judgement, on concern for the animal, on knowledge of animal behavior and animal husbandry, and on familiarity with the species. Investigators working with species unfamiliar to them should obtain all pertinent information before confining those animals. It also may be necessary to test and compare several methods of housing to determine the most appropriate one for the well-being of the animal and the purpose of the study. Findings should be part of a permanent record system and animal logbook associated with the study and the maintenance facility.

Housing

Housing for wild vertebrates should approximate natural conditions as closely as possible. Housing should provide safety and comfort for the animal as well as meet the study objectives. Methods of housing should provide for behavioral needs, safety, adequate exercise and rest, and conditions for the general well-being of the animal. Considerations depend on the animal involved and include isolation or refuge areas, natural materials, dust and water baths, natural foods, sunlight, and fresh air. Housing should incorporate as many aspects of natural living as possible, such as brushy areas for escape, resting cover, shade and protection from environmental elements, a natural stream traversing the pen, rocky areas for hoofed animals that need to wear down their hooves, and social groups of animals kept together. Housing of compatible species in a common pen also will provide for social interaction. Frequency of cleaning should be a compromise between level of cleanliness necessary to prevent disease and amount of stress imposed by cleaning (Fig. 6.8).

In general, housing must be of adequate size to allow for the physical and behavioral needs of the animals, while allowing scientists to collect appropriate data. For many housing situations, the pen can be large and natural, with a smaller internal or attached catch pen to restrain animals for experimental techniques. Pen construction materials must provide for the safety of the animals, as well as prevent the animals from escaping. Materials should be of sufficient durability to last for the intended period of confinement. When long-term confinement (weeks or longer) is necessary, or pens are to be reused, materials with impervious surfaces should be used to facilitate sanitation and minimize the potential for survival of animal pathogens. All animals that are inherently dangerous, are environmentally injurious, or have a propensity for escape require special attention. Double walls or

Photo by Milton Friend

Figure 6.8 *A high quality enclosure for New Zealand black stilt that approximates several aspects of the natural habitat and provides safety and comfort for the birds.*

double enclosures, covered tops of enclosures, and construction with metal bars or chain link may be required, depending on the species. Mesh size and spacing between fencing materials must be small enough to prevent the head of an animal from extending through the fence. Smaller fencing mesh also is more visible to animals. Colored flagging material may be necessary for animals to visualize fencing until they become accustomed to it. Animals should be released into the housing in a calm and unstressed manner so that initial mortality and morbidity from fence encounters are minimal. A small dose of tranquilizer often will reduce the immediate flight response when an animal is released into the housing and may help prevent initial injuries. Once animals have investigated the limits of the housing, injury occurrence is minimized if investigators do not cause undo flight reactions.

Adequacy of housing often can be judged on normal behavior patterns, weight gains and growth, survival rates, reproductive success, and physical appearance of the animals involved in the research project. Established guidelines for housing laboratory and farm animals were provided by the Canadian Council on Animal Care (1980, 1984). Additional guidelines for housing requirements of fish, amphibians reptiles, wild birds, and small mammals were reported by the appropriate professional societies and appear in the Animal Welfare Act (see also “Professional society guidelines” at the end of this chapter).

Nutrition

Nutrition must meet the needs of the animal unless deviations are an approved purpose of the investigation. Researchers are responsible for determining the appropriate nutritional needs of study animals prior to placing them in confinement and for obtaining adequate food supplies to sustain the animals during the period of confinement. Feeding and watering should be under the direct supervision of an individual

trained and experienced in animal care for the species being maintained. Animal care personnel must be familiar with the animals being studied so abnormalities in appearance and behavior that may be indicative of nutritional deficiencies can be recognized quickly.

Transportation

General Considerations

A variety of vehicles such as conventional motor vehicles, all-terrain vehicles, snow machines, rotary and fixed-wing aircraft, and boats are used to transport wild animals. The species involved, method of transportation selected, and length of time an animal is to be transported are important factors regarding the type of care and conditions of containment required to maintain the animal in a state of well-being (Fig. 6.9). To the extent possible, selection of transportation vehicles should take into account maintenance of the animal in a comfortable environment. Veterinary assistance may be required to prescribe and administer appropriate tranquilizers or other drugs when conditions of transportation are likely to result in a high level of stress to the animal due to its behavioral and physiological characteristics, restrictions of confinement, engine noise, and rigors of the trip. The transportation process should be as brief as possible. This can be expedited by proper and adequate planning to assure that transportation vehicles and housing units in appropriate numbers and size are available and ready for use as needed; that food, water, bedding, and other needs to provide for the animals also are available; that individuals involved in the transportation process are trained in the procedures to be used in containment and transportation of the

Photos by Julie Langenberg, International Crane Foundation

Figure 6.9 ***(A)*** *Restraint of bighorn sheep being translocated via helicopter. The legs have been immobilized to prevent injury to the animal and holders.* ***(B)*** *Blinders on this caribou reduces stress from the presence of humans. Legs are restrained similar to the procedure shown for the bighorn sheep.*

animals; and that all permits, health certificates, and other paperwork have been completed to the extent possible.

When interstate movement of animals or shipment by commercial carriers is involved, scheduling of transportation segments to minimize the number of transfers and delays between transfers, having someone involved with the project meet the shipment at each transfer point, and, when appropriate, arranging for prompt clearance of animals by veterinary and customs inspectors can result in major reductions in transit time. The receiving party should be on-site when the animals reach their destination.

For some species, periodic rest periods are required to allow the animals to feed undisturbed. Other species are best transported when they are normally inactive and do not feed. Ventilation within the housing unit and transportation vehicle should provide for adequate air movement to keep animals comfortable and avoid buildup of exhaust gases. Subdued lighting and visual barriers between animals and humans and between animals and their transportation environment should be provided to help keep the animals calm. The U.S. Fish and Wildlife Service has published rules for the Humane and Healthful Transport of Wild Animals and Birds to the United States (see Fed Reg. 50 CFR Part 14).

Confinement During Shipping

Animal containers should be inspected to assure they have no sharp edges, protrusions, or rough surfaces that could cause injury during transport. When appropriate, containers also should be padded to help prevent injury. The floor of shipping containers should allow reasonable footing to prevent falling due to a slippery surface. Also, containers should not have coatings or be constructed of materials that are toxic and could be consumed by the animal through licking or chewing during transportation. In general, housing units of porous materials, such as cardboard boxes, should not be reused; all other containers used to house animals should be suitably disinfected between uses (Fig. 6.10). That portion of the transportation vehicle used to contain the housing units also should be disinfected.

Grouping or separation of animals being transported at the same time should take into consideration the species, age, and other appropriate factors. Direct contact generally should be maintained between females and their dependent young, particularly if abandonment may result (unless the young are to be maintained by some other means). Birds should be isolated in separate cells within the shipping container; if this cannot be done, each individual should have sufficient space to assume normal postures and engage in comfort and maintenance activities unimpeded by other birds (Ad Hoc Committee on the Use of Wild Birds in Research, 1988).

Health Aspects

For short-term transportation (less than 30 min), basic considerations are to prevent pain, injury, and undue stress. Thermoregulation capabilities of the species must be considered when an animal is removed from its existing environment and placed in the transportation environment. Transported animals should be protected from exposure to inclement weather, harsh environmental conditions, and major temperature fluctuations and extremes.

Bedding, feed, and water should be provided, as appropriate, and the animals should be observed periodically to determine their state of well-being during transportation. On-site veterinary assistance may be warranted to monitor animals and to provide life-support assistance should a medical emergency occur during transportation or at the release or field study site. Selection of veterinary assistance should focus on the individual's knowledge and experience with the wildlife species involved. Any animals that die during transit should be removed as soon as practical from the sight and olfactory detection of other animals being transported. These carcasses should be retained for pathological examinations regarding cause of death. Similarly, animals that become severely injured or clinically ill should be removed and responsibly euthanized. Euthanasia should not take place in the presence of other live animals. Sick animals disposed of in this manner also should be retained for pathological assessments. Determinations of cause of death are needed to assess whether the remaining animals are at risk from pathogens associated with the dead animals.

Surgical and Medical Procedures

Guidelines for wildlife medical procedures

Wildlife field research can involve surgical and medical procedures such as implanting radio transmitters and surgical sex determination in birds. Incorporation of such techniques into a research protocol should follow these guidelines:

1. Surgical and medical techniques used should be based on accepted protocols for the studied species or for the most closely related domesticated species. The Canadian Council on Animal Care's (1984) Guide to the Care and Use of Experimental Animals, Volume 2, is a good source of such information.

2. Protocols should be developed and, if possible, implemented in collaboration with a qualified veterinarian. Only properly trained personnel, conversant in all techniques necessary, should conduct the procedures.

3. Protocols must be reviewed carefully by the ACUC with special attention paid to limiting pain during the actual procedure and post-procedure period.

4. Adequate anesthesia and/or analgesia must be provided.

Photo by Milton Friend

Photo by Milton Friend

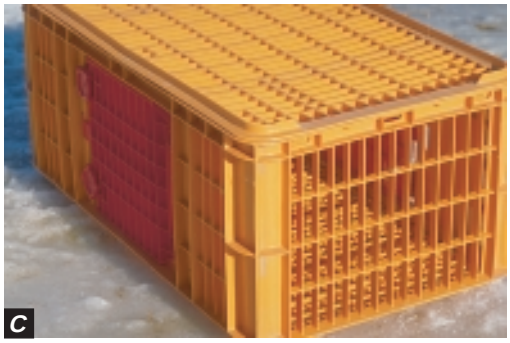


Photo by James Runningen

Photo by Louis Sileo

Figure 6.10 ***(A)*** *Canada geese restrained within burlap bags with openings for the head and neck for short-distance transportation by vehicle.* ***(B)*** *Porous materials such as these bags and the cardboard boxes these Hungarian partridge are being released from should not be reused for animal transport. More permanent holding containers such as* ***(C)*** *plastic poultry crates and* ***(D)*** *large animal crates should be thoroughly washed and disinfected between uses.*

Minor Procedures

Minor medical procedures such as collection of blood, administration of drugs intravenously or intramuscularly, biopsies of superficial structures such as skin, and sutured attachment of radio transmitters usually can be performed safely and responsibly in the field without complicated equipment. However, it is the researcher's responsibility to choose the least invasive and least painful technique, minimize the duration of the procedure, use the most appropriate equipment and aseptic technique, and provide analgesia or sedation when indicated.

Major Procedures

As defined by the Animal Welfare Act, major operative procedures are (p. 36,121) "any surgical intervention that penetrates and exposes a body cavity or any procedure which produces permanent impairment of physical or physiological functions." Major surgical procedures, when survival of the animal is intended, should be performed only under proper anesthesia and with sterile technique. Examples of major procedures used in wildlife research include laparotomy, surgical flight restraint, and sterilization. These procedures should be performed only in a clean space set aside for sterile surgery, with surgical instruments and drapes of the proper type, and with anesthesia protocols judged to be safe and responsible for the species involved. Necessary equipment and trained personnel to deal with surgery or anesthesia-related emergencies (i.e., severe blood loss, cessation of breathing or cardiac function, severe hypo- or hyperthermia, acid-base imbalances) should be available at all times. This will maximize the success and subsequent scientific return from those often costly procedures and, therefore, minimize the number of animals needed and amount of animal distress (Fig. 6.11).

Photo by J. Christian Franson

Figure 6.11 *Invasive surgical procedures should be done only by properly trained personnel knowledgeable of techniques necessary to successfully carry out the procedure and appropriately respond to medical emergencies that might arise.*

Medical Considerations

Wildlife field researchers should have access to veterinary consultation and take responsibility to prepare themselves to deal with any health problems that might arise in their study population. Sometimes intervention and control of a natural disease process may not be advisable and may interfere with the study's goals. However, if the health problem arises due to the researcher's work, or if it will interfere with the study, the researcher must be ready to respond. Preparations should include gaining familiarity with the common diseases and health problems of the species under study, establishing a contact with a veterinary consultant, and having appropriate treatment or control equipment and drugs on hand or easily accessible. The researcher also is responsible for evaluating the possible impact of disease in the study animals on the larger population or ecosystem as a whole, and for making the maintenance of their welfare a priority as decisions are made. This is especially true when release or translocation of animals is part of a study; disease must be considered in evaluating the advisability of the program.

Euthanasia

Euthanasia is defined under the Animal Welfare Act as (p. 36,121) "the humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death." Euthanasia may not be an approved component of a field study, but it may become a necessary health care option in a study involving capture, restraint, or surgical procedures. Therefore, all wildlife researchers involved in invasive studies must be familiar with the approved euthanasia methods for their study species (Andrews and others, 1993) and have the appropriate equipment/drugs on hand so euthanasia can be performed quickly.

Disease Considerations

Field investigators need to be fully aware of disease concepts so they may avoid introduction of new disease problems into animal populations or the spread of disease to other populations and locations as a result of their studies. Disease introductions and spread occur as a result of animals brought to the field research site to serve as biological sentinels, as decoys to lure and capture other animals, for species introductions or releases to supplement existing populations, for behavioral studies, for assistance in tracking or retrieving animals, and for other purposes. All of these uses of animals involve acceptable methods for scientific research and wildlife management. However, under no circumstances should the well-being of free-ranging wildlife populations be unduly jeopardized by disease risks associated with animal use in field research. Field investigators have ethical and

professional obligations to take appropriate actions for minimizing the introduction of the following: (a) new disease agents, (b) vectors (e.g., ticks and internal parasites) capable of efficiently transmitting indigenous, dormant diseases or those not currently being effectively transmitted, and (c) species that can serve as amplification hosts for transmitting indigenous diseases to other species (Fig. 6.12).

In addition, animals that are highly susceptible to diseases indigenous to the study location should not be released into the wild without using applicable prophylactic measures, unless these animals are to serve as biological sentinels for disease investigations. Biological sentinels should be monitored closely and euthanized by approved, responsible methods as soon as is practical after study objectives have been met.

Disease introduction and spread can result from mechanical means such as contaminated personnel, supplies, and equipment in addition to the biological processes identified above. Steps taken to address disease prevention are far more cost effective than disease control activities initiated after a problem has developed.

Photo by Milton Friend

Figure 6.12 *Wildlife are often referred to as a "biological package" as the relocation of animals may involve life forms other than the animals themselves. The ticks feeding on this velvet covered antler could be disease carriers. Once introduced into a new area, the ticks may become an important vector for transmission of an indigenous disease. Disease potential is an important consideration that should be adequately addressed when translocating wildlife.*

Wildlife disease prevention during field research

Protection of free-ranging wildlife from disease is aided by the following actions:

1. Appropriate health certification should be required for all animals being brought to the site of field investigations. State veterinary officials should be contacted to determine what specific testing must be done when animals are moved into their jurisdiction.

2. Appropriate disinfection procedures should be used for investigators and their equipment when disease risks are present.

3. Prior knowledge of disease activity at the study site should be obtained to guide actions involving the research study.

4. Source for any animals being brought to a field investigation site (captive-reared and relocated wild stock) should be evaluated for inherent disease problems, and appropriate steps should be taken to avoid disease introductions.

5. To the extent possible, animals should be held under surveillance for 15–30 days prior to their release into the wild, and only healthy animals should be released. These animals should not be mixed with other species during transportation and should be isolated from other animals during the surveillance period.

6. Any animals that die should be examined by a disease diagnostic laboratory having competency for determining cause of death in the species involved; these findings should be used to guide appropriate actions (Fig. 6.13).

7. Animals that become clinically ill should be examined by disease specialists, and their counsel should be used to protect the well-being of other animals within the study area.

Photo by James Runningen

Figure 6.13 *Timely diagnosis of causes of wildlife morbidity and mortality is invaluable for the detection of emerging hazards that can jeopardize the well-being of the population being studied and may be of great potential consequences. Submission of animals that die to competent laboratories provides information useful for intervention.*

Animal Disposition at Completion of Study

When live animals are in the possession of investigators or under their control at the time of study completion, an evaluation must be made as to whether these animals can be released to a free-ranging existence, should be maintained under controlled conditions, or should be euthanized.

Animal release guidelines

As a general rule, field-captured animals should be released only:

1. At the site of the original capture, unless conservation efforts or safety considerations dictate otherwise. Prior approval for releases at noncapture sites should be obtained from appropriate State/Federal agencies. Relocation release sites should be within the native range of the species, or established range for introduced species, and be in habitat suitable for species survival;

2. When the released animal can be reasonably expected to function normally within the population;

3. When local and seasonal conditions are conducive to survival;

4. When the ability to survive in nature has not been irreversibly impaired; and

5. When release is not likely to spread pathogens or contribute to disease processes in other ways.

The decision of whether to release captive-reared animals into the wild after completion of a field research project demands more rigorous evaluation than for field-captured animals. In addition to evaluating the future well-being of the animal being released, impacts on other animals of the same species and competition and risks for other species sharing that environment also must be considered. Rarely, if ever, will releases of captive-reared animals at the completion of research studies be justified on the basis of animal welfare considerations.

When animals are to be released, efforts should be made to enhance their chances of survival. Animals should be in good physical condition and released when weather conditions are favorable, at a time of day when they are able to locate food and cover that meet survival needs.

Animals that cannot be released should be considered for distribution to other scientists for further study. However, if the animal was subject to a major invasive procedure, it may not be appropriate for additional experimentation. Animals not suitable for research may be suitable display animals that can be donated to a zoo or other type of educational institution.

When animals must be euthanized, responsible methods appropriate for the species and circumstances must be used. Care must be taken to assure that the animal is dead before disposal of the carcass. Also, disposal procedures must prevent carcasses containing toxic substances or drugs from the research investigations or euthanasia procedures to enter the food web of other animals. To the extent feasible, euthanized animals should be properly preserved and used as voucher specimens or for teaching purposes.

Safety Considerations

Researchers working with free-ranging wildlife are subject to enhanced levels of exposure to wildlife diseases transmissible to humans. Disease transmission may involve direct contact with infected animals such as those with rabies, contact with disease vectors such as ticks transmitting Lyme disease, or contact with contaminated environments such as bird roosts harboring histoplasmosis. Field investigators should become familiar with the common diseases of wildlife species they are working with and the relative prevalence of those diseases in the populations they are studying. Consultation with a physician regarding immunization or other preventative treatment is advised when serious diseases for humans commonly occur in the populations being studied. Investigators who become ill should seek medical assistance and advise their physicians of their exposure to potentially hazardous animals, diseases, and environmental conditions.

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Sources of assistance for technical information, implementation, and interpretation of the Animal Welfare Act

Animal Welfare Information Center
National Agricultural Library
10301 Baltimore Ave., 5th Floor
Beltsville, MD 20705–2351
(301) 504-6212
fax (301) 504-7125

National Library of Medicine
8600 Rockville Pike
Bethesda, MD 20894
(301) 594-5983

Scientists Center for Animal Welfare
7833 Walker Dr., Suite 340
Greenbelt, MD 20770–3229
(301) 345-3500

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
4700 River Rd.
Riverdale, MD 20737
(301) 734-7833